VOL. 48, NOS. 3 & 4 NOVEMBER 1958

NEMATODE PARASITE POPULATIONS IN SHEEP ON LOWLAND FARMS

IV. THE EFFECTS OF ANTHELMINTIC TREATMENT

BY H. D. CROFTON

Department of Zoology, University of Bristol

(With 7 Figures in the Text)

CONTENTS

		PAGE
I.	Introduction	235
II.	Materials and methods	236
III.	Results	236
	(*a*) Monthly treatment with phenothiazine	236
	(*b*) Monthly treatment with copper and nicotine sulphate mixture	236
	(*c*) Differences in dosing intervals	237
	(*d*) After-treatment effects	238
IV.	Discussion	239
V.	General conclusions and their application	240
	References	242

I. INTRODUCTION

In the last twenty-five years, since it was realized that the control of flock infections was more important than the treatment of individuals with patent infections, there has been an increasing interest in the use of anthelmintic drugs. As might be expected there has been a wide search for the most effective chemicals and considerable research into the best methods of administration, and as a consequence of this many workers have been engaged in devising methods of testing the efficiency of various compounds.

For the selection of drugs from the wealth of chemicals available, various *in vitro* tests have been described (e.g. Baldwin, 1943; Chance & Mansour, 1949; Krotov, 1953, 1956; Leiper, 1952; Parnell, 1936). Mackie & Parnell (1955) compared some of these methods, and showed that there was considerable variation in the results obtained from different tests—a conclusion which might be deduced from the work of Alexander & Trim (1946) and Trim (1944, 1949). Other workers have used *in vivo* tests, basing them mainly on Hall's *Index of Efficiency*. The most satisfying method of bio-assay is that described and discussed by Whitlock (1943) and Whitlock & Bliss (1943).

On a different scale there has evolved the field trial. A great impetus was given in this direction by the work of Peters, Leiper & Clapham (1941), who described what must be regarded as the basic technique for this type of test.

All of these studies have been directed at the evaluation of the efficiency of drugs against parasites without regard to the dynamics of the parasite–host relationship. Admittedly, the determination of anthelmintic potency is a basic

requirement, but the efficiency of anthelmintic treatments in flock animals is affected by many factors other than parasiticidal competence of the drugs used.

Obviously the effectiveness of any series of treatments must be dependent upon the rate of recruitment of parasites between doses, and the present work is an attempt to analyse the effects of drug treatments on parasite populations.

II. MATERIALS AND METHODS

Observations were made on commercial flocks. No attempt was made to influence the owner in his choice of drug or in the method of administration, except that where proprietary drugs were used recommendations were made to ensure that each dose was within the therapeutic range advised by the manufacturer. Whenever there was any doubt about the instructions supplied with the product the dose was adjusted to conform with accepted practice. For example, in the case of preparations of phenothiazine, no lamb received a dose of less than 15 g.

The interval between treatments was agreed before the beginning of the observations, but otherwise the farmer adhered to his normal practice and changes of pasture followed the usual routine of the farm. So far as it was possible to ensure, every lamb in a flock was treated at each dosing occasion. No control groups were left. For purposes of general comparison, observations were made on a flock which received no anthelmintic treatment.

Assessment of the results of treatment was dependent upon worm egg counts, these being made using the methods described in previous papers.

III. RESULTS

As a method of presenting the raw data, mean egg counts are plotted for each flock (see Figs. 1, 3, 5 and 7). For more detailed discussion the counts have been plotted on a logarithmic scale and a regression line calculated for each interval between treatments. In each case a regression line for the pre-treatment period is also given.

(*a*) *Monthly treatment with phenothiazine*

Figs. 1 and 2 summarize the results obtained from a flock of Cross Clun lambs (flock A), which were dosed at monthly intervals from June to August. The ewes were treated at the same time as the lambs. It must be noted that the after-treatment regression lines plotted in Fig. 2 are each based on only four sets of egg counts. The slopes of the lines are fairly constant. The efficiency of the phenothiazine as calculated from egg counts before and after treatment for each occasion varied from 74 to 78 %. Although the lambs harboured a mixed infection of worms the most numerous were *Ostertagia* spp.

(*b*) *Monthly treatment with copper and nicotine sulphate mixture*

A similar flock of Cross Clun lambs (flock B), dosed at monthly intervals with copper and nicotine sulphate mixture, was observed. In this case only the lambs were treated. The results are shown in Figs 3 and 4. Again the regression lines are

based on a small number of readings, but the slopes are fairly constant. The efficiency of the mixture as calculated from before and after treatment egg counts varied from 41 to 44%. The most numerous worms were *Ostertagia* spp.

(c) *Differences in dosing intervals*

Two other flocks (flocks C and D) dosed with phenothiazine were also observed. The amount given at each treatment was the same as that given to lambs in flock A, but in the case of flock C the treatment was given fortnightly, and in the case of

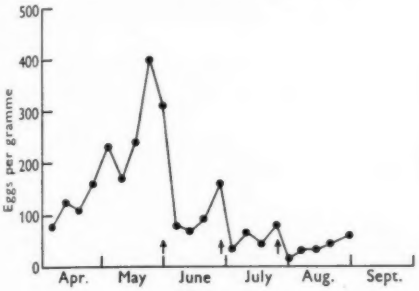


Fig. 1

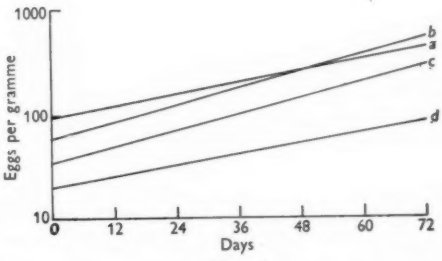


Fig. 2

Fig. 1. Flock A: monthly treatment with phenothiazine. Worm egg counts, April–September.

Fig. 2. Regression lines calculated for before and after treatment egg counts (flock A).
a (before treatment): $\log y = 2{\cdot}258 + 0{\cdot}008\ (\bar{x} - x)$.
b (after first treatment): $\log y = 1{\cdot}982 + 0{\cdot}014\ (\bar{x} - x)$.
c (after second treatment): $\log y = 1{\cdot}734 + 0{\cdot}012\ (\bar{x} - x)$.
d (after third treatment): $\log y = 1{\cdot}437 + 0{\cdot}008\ (\bar{x} - x)$.

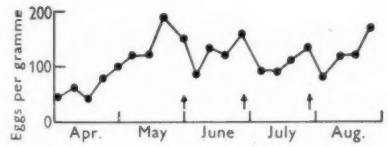


Fig. 3

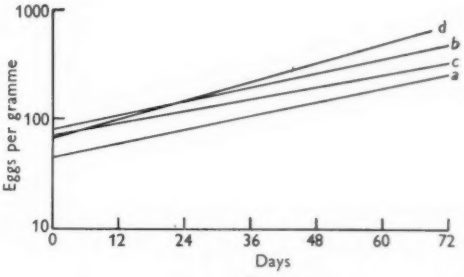


Fig. 4

Fig. 3. Flock B: monthly treatment with copper and nicotine sulphate mixture. Worm egg counts, April–September.

Fig. 4. Regression lines calculated for before and after treatment egg counts (flock B).
a (before treatment): $\log y = 1{\cdot}961 + 0{\cdot}011\ (\bar{x} - x)$.
b (after first treatment): $\log y = 2{\cdot}088 + 0{\cdot}011\ (\bar{x} - x)$.
c (after second treatment): $\log y = 2{\cdot}024 + 0{\cdot}009\ (\bar{x} - x)$.
d (after third treatment): $\log y = 2{\cdot}076 + 0{\cdot}014\ (\bar{x} - x)$.

flock D at 6-week intervals. The results are plotted in Figs. 5A, B and 6. It must be noted that regression lines for flock C were not calculated because of inability to provide sufficient readings between treatments. The efficiency of the individual treatments varied only slightly (flock D, 62–77%).

(d) After-treatment effects

Observations were continued after the final treatment in the case of all flocks. Egg counts for each flock during autumn are recorded in Fig. 7. Plotted on the same scale are egg counts for flock E to which no treatment had been given. It will be seen that in the untreated flock the autumn counts were very low, this being the result of a spontaneous recovery of a type described in a previous paper (Crofton, 1955). In the treated flocks the post-treatment counts were higher than in the un-

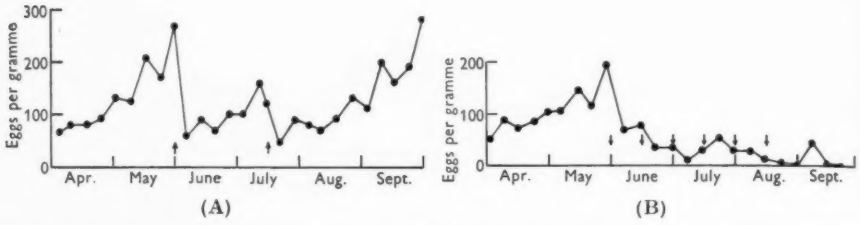


Fig. 5. (A) Flock D: 6-weekly treatment with phenothiazine. Worm egg counts, April–September. (B) Flock C: fortnightly treatment with phenothiazine. Worm egg counts, April–September.

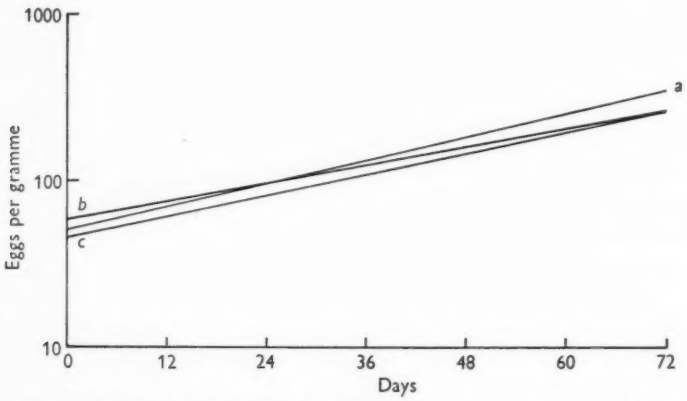


Fig. 6. Regression lines calculated for before and after treatment egg counts (flock D).
a (before treatment): $\log y = 2{\cdot}089 + 0{\cdot}0105\ (\bar{x} - x)$.
b (after first treatment): $\log y = 1{\cdot}983 + 0{\cdot}008\ (\bar{x} - x)$.
c (after second treatment): $\log y = 2{\cdot}078 + 0{\cdot}010\ (\bar{x} - x)$.

treated flock. That this difference is a real one is suggested by the highest counts occurring in the flocks which had undergone most frequent treatment previously. Also a comparison of flocks treated with phenothiazine and copper and nicotine sulphate mixture shows that the autumn counts were lower in the flock treated with the latter anthelmintic, which otherwise appeared to be less efficient (see also Gordon, 1945, 1948).

IV. DISCUSSION

The results presented show that under normal conditions of monthly treatment with either phenothiazine or copper and nicotine sulphate mixture the worm egg counts in sheep flocks were reduced. This reduction, however, was transient and the general pattern of the rate of increase of worm remained unaltered, so that

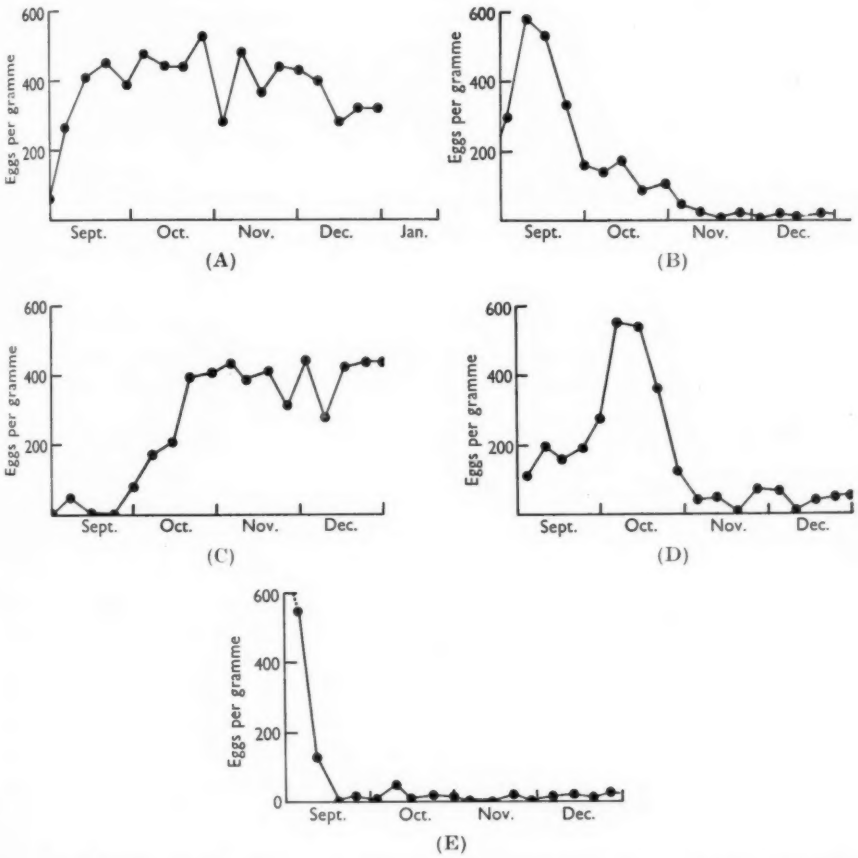


Fig. 7. (A) Flock A: egg counts in autumn after monthly treatment with phenothiazine. (B) Flock B: egg counts in autumn after monthly treatment with copper and nicotine sulphate mixture. (C) Flock C: egg counts in autumn after fortnightly treatment with phenothiazine. (D) Flock D: egg counts in autumn after 6-weekly treatment with phenothiazine. (E) Flock E: egg counts in autumn after no treatment.

treatment merely delayed the attainment of the full parasite potential. In fact we may interpret the results as showing that such anthelmintic treatment is merely a method of postponing the rise in worm egg count without altering the intrinsic rate of multiplication of the parasite. The temporary checks to the build-up of the parasite population might be expected if the drugs concerned had effect on some

but not all stages (see Gordon, 1939, 1940, 1943). That the treatment had no marked effect on the main parasite population (the immature and the free living stages) can be deduced from the fact that if the infection pressure had been altered, the apparent generation time, and therefore the slope of the rate of increase, would have changed.

Treatment given at intervals of 6 weeks showed similar effects. The main difference was that the fewer treatments caused a smaller total delay in the increase.

Fortnightly treatment was too frequent to permit regression lines to be fitted, but it is apparent from the general trend that a change in the rate of increase must have occurred. This change might be due to a change in the fecundity of the parasites or by an increase in the effective generation time. While there is some evidence to suggest that phenothiazine may affect egg-laying rates by producing temporary or partial sterility in surviving worms, this is normally only a transient effect and is not really an effect on the intrinsic fecundity of the parasite population. We may conclude that the change of pattern of increase is more likely to be due to a change in the effective generation time caused by a reduction in the probability of successful encounter between parasite and host.

In the flock treated with copper and nicotine mixture the efficiency of the individual treatments was obviously lower than that of a phenothiazine treatment. The series of monthly treatments had a delaying effect on the rise of the worm egg counts and they never reached a dangerous level. It is interesting to speculate, however, on the ultimate fate of the flock if the infection pressure had been higher initially.

Apart from the effects during treatment it is interesting to consider the course of the infections in flocks after September. In an untreated flock (Fig. 7), after the worm egg counts had reached a high level in September they fell rapidly and remained low as has been described and discussed in a previous paper (Crofton, 1955). In the same paper preliminary observations of flocks treated with anthelmintics were recorded. The present work presents more data on this subject and supports the suggestion that reduction of infection by anthelmintic treatment results in a poorer stimulation of the host, which consequently does not produce a fully developed immunity response. It has been shown that there is some positive relationship between the degree of efficiency of the actual treatment and the prolongation of the autumn infection. Undoubtedly the relationship would be more marked in the flock treated fortnightly if the previous treatment had not had some effect on the infection pressure.

V. GENERAL CONCLUSIONS AND THEIR APPLICATION

We may conclude that monthly treatments with both phenothiazine and copper and nicotine sulphate mixture effectively delay the rise in egg counts in sheep flocks. With an infection pressure of the same order as that in the observed flocks both drugs appear to exert an adequate control. With an increased infection pressure brought about by over-stocking or by any other means the copper and nicotine sulphate mixture might provide less adequate control than phenothiazine, and an outbreak might occur. The index of efficiency of these drugs is assessed as the effect of single treatments, but it is better to consider their efficiency

in relation to the infection pressure or rate of increase of the population. This might be done by thinking in terms of a composite factor—the 'Delaying Time'. This factor would vary for different flocks even with the same drug. It would be dependent on the pattern of increase of flock infection. If the rate of increase was low for parasites of a given flock, then the 'Delaying Time' of each drug treatment would be increased so that adequate anthelmintic treatment could be provided by using a less costly and less efficient drug or by lengthening the intervals between treatments with the more efficient one.

The work suggests that the interval between treatments could vary according to the particular requirements of the flock and that the general rule of monthly dosing is based upon a fallacy. The choice of monthly treatment has been made in the past because the majority of the more pathogenic forms complete their life history in approximately this period. The only condition under which this choice could be justified would be that in which the anthelmintic killed all stages of the parasites within the host. No known anthelmintic is completely efficient against mature worms and few have any effect on the immature stages. Fortnightly treatment with phenothiazine had a great effect on the worm population of the flock studied. It appeared to produce a real decrease in the infection pressure and not merely a delay in the population rise. It must be realized, however, that fortnightly treatment might not produce this effect in other flocks if the infection pressure started at a higher level.

Unfortunately the only method of determining the infection pressure is by intensive study of each individual flock and this is not a practical proposition except under experimental conditions.

We may, however, approach the problem in a different way. If a farmer normally ensures adequate control by giving four treatments at monthly intervals, then it can be deduced from the present work that the same control would be achieved by giving four treatments at shorter intervals. Let us suppose that the interval chosen was fourteen days, then the total delaying time would be the same. He might, however, achieve an additional effect on the parasite potential by fortnightly treatment, as occurred in the observations described above. By treating the flock early in the season he might not only reduce the labour in handling and the size of each dose, but might also make a real reduction in the parasite population of his farm. Obviously further work must be done before any such recommendation could be made.

The danger of anthelmintic treatment has been illustrated by the observations on the flocks after treatment. Undoubtedly the worm egg output in treated flocks is higher in autumn than in flocks which have been untreated and have thrown off their infection spontaneously. This increase in output might be of considerable importance because the time of survival of infective stages is much longer then than in the summer period. The net result of treatment may be to produce flocks which remain susceptible in the autumn and winter to infections from which they may later die. It is certainly true that many farmers whose hoggs are affected by outbreaks of winter trichostrongylosis claim that the sheep have had more than adequate anthelmintic treatment during their period as lambs.

Finally, although this paper has been exclusively concerned with anthelmintics the author agrees with the statement of Whitlock (1955)—'they [anthelmintics] are probably the least important part of an adequate parasite control program'.

The author is indebted to the Agricultural Research Council for financial assistance in the form of a special research grant, and for the technical assistance of Mr E. J. Sands.

REFERENCES

ALEXANDER, A. E. & TRIM, A. R. (1946). The biological activity of phenolic compounds. The effect of surface active substances upon the penetration of hexyl resorcinol into *Ascaris lumbricoides* var. *suis*. *Proc. Roy. Soc.* B, **133**, 220–34.

BALDWIN, E. (1943). An *in vitro* method for the chemotherapeutic investigation of anthelmintic potency. *Parasitology*, **35**, 89–111.

CHANCE, M. R. A. & MANSOUR, T. E. (1949). A kymographic study of the action of drugs on the liver fluke. *Brit. J. Pharmacol.* **4**, 7–13.

CROFTON, H. D. (1955). Nematode parasite populations in sheep on lowland farms. II. Worm egg counts in lambs. *Parasitology*, **45**, 99–115.

GORDON, H. McL. (1939). Anthelmintic efficiency against immature *Haemonchus contortus*. *Aust. Vet. J.* **15**, 57–66.

GORDON, H. McL. (1940). The anthelmintic efficiency of phenothiazine against immature *Haemonchus contortus*. *J. Coun. Sci. Industr. Res. Aust.* **13**, 245–6.

GORDON, H. McL. (1943). The anthelmintic efficiency of phenothiazine against immature *Trichostrongylus* spp. in sheep. *J. Coun. Sci. Industr. Res. Aust.* **16**, 1–4.

GORDON, H. McL. (1945). Phenothiazine as an anthelmintic. *Aust. Vet. J.* **21**, 90–95.

GORDON, H. McL. (1948). The epidemiology of parasitic diseases with special reference to studies with nematode parasites of sheep. *Aust. Vet. J.* **24**, 17–45.

KROTOV, A. I. (1953). (A study of the therapy of ascaridosis. A first communication on a method of studying the reactions of Ascaris in anthelmintic preparations *in vitro*.) *Med. Parasit., Moscow*, **22**, 387–95.

KROTOV, A. I. (1956). (Data on the physiology of the motor responses of *Ascaris*.) *Med. Parasit., Moscow*, **25**, 58–60.

LEIPER, J. W. G. (1952). The testing of anthelmintics with particular reference to the development of new agents. *Vet. Rec.* **64**, 438–9.

MACKIE, A. & PARNELL, I. W. (1955). A comparison of the results of four *in vitro* anthelmintic testing techniques. *J. Pharm. Pharmacol.* **7**, 416–20.

PARNELL, I. W. (1936). Studies on the bionomics and control of the bursate nematodes of horses and sheep. II. Technique. *Canad. J. Res.* (Sect. D), **14**, 71–81.

PETERS, B. G., LEIPER, J. W. G. & CLAPHAM, P. A. (1941). A phenothiazine experiment statistically treated. *J. Helminth.* **19**, 9–24.

TRIM, A. R. (1944). Experiments on the mode of action of hexyl-resorcinol as an anthelmintic. *Parasitology*, **35**, 209–19.

TRIM, A. R. (1949). The kinetics of the penetration of some representative anthelmintics and related compounds into *Ascaris lumbricoides* var. *suis*. *Parasitology*, **39**, 281–90.

WHITLOCK, J. H. (1943). Characteristics of the population available for bioassay of anthelmintics in *Nippostrongylus muris* infection in albino rats. *J. Parasit.* **29**, 42–7.

WHITLOCK, J. H. (1955). Trichostrongylidosis in sheep and cattle. *Proc. Book, Amer. Vet. Med. Ass.*, 92*nd Annual Meeting* 1955, pp. 123–31.

WHITLOCK, J. H. & BLISS, C. I. (1943). A bio-assay technique for anthelmintics. *J. Parasit.* **29**, 48–58.

(*MS. received for publication* 30. IV. 1957.—Ed.)

NEMATODE PARASITE POPULATIONS IN SHEEP ON LOWLAND FARMS

V. FURTHER OBSERVATIONS ON THE POST-PARTURIENT RISE AND A DISCUSSION OF ITS SIGNIFICANCE

By H. D. CROFTON

Department of Zoology, University of Bristol

(With 6 Figures in the Text)

CONTENTS

		PAGE
I.	Introduction	243
II.	Materials and methods	244
III.	Results	244
	(1) Autumn-lambing flocks	244
	(2) Spring-lambing flock	245
	(3) Spring- and autumn-lambing flock	245
	(4) Rises in non-reproductive sheep	246
IV.	Discussion	246
	References	250

I. INTRODUCTION

In a previous paper (Crofton, 1954) it was shown that the rise in egg counts occurring in ewe flocks in spring was related to the time of lambing. It was further shown that the rise in individuals occurred about 6 weeks after parturition and was normally of short duration. While the mean time of increased egg count for each individual was about 2 weeks, the increased egg count in the flock was spread over a period equivalent to the length of the lambing period. It is clear that the apparent rise in the flock as a whole will be greatest when all the flock lambs within a short period. With greater scatter of times of parturition the flock rise will be smaller but will extend over a greater period. This association of rise in egg count with time of parturition is obviously one of considerable selective advantage, in so far as an increase in infective stages will occur at a time when the susceptible lambs have a reduced milk intake and an increased grass intake. The synchronization of availability of infective stages and the occurrence of susceptible individuals is further emphasized by the relation of the period of increase to the time of birth of the lambs, the duration of the period of increase in the flock being directly proportional to the duration of the lambing period. This type of 'escapement' control is seen in so many other host-parasite relationships that it makes considerable appeal on general grounds. Other workers, Morgan, Parnell & Rayski (1951), have, however, related the rise in egg counts to climatological factors, but climatic control would not give the close synchronization described above.

Although the evidence presented previously (Crofton, 1954) is convincing as far as it goes, the data given related to spring-lambing flocks only. In the present paper flocks lambing at other times of the year are considered.

II. MATERIALS AND METHODS

Observations were made on a series of flocks lambing at different times of the year. The flock referred to as flock A in this account lambed in autumn and consisted of Dorset Horn sheep. Flock B lambed in the spring and the ewes were Cluns. Flock C lambed in the spring and the autumn and consisted of older Dorset Horn ewes which were lambed for a few seasons and then replaced.

In all cases samples of faeces were collected fortnightly or weekly according to circumstances and season. Egg counts were made as described (Crofton, 1954).

III. RESULTS

(1) *Autumn-lambing flocks*

(*a*) *Flock A Ewes*

Fig. 1. summarizes the egg-count data obtained by sampling the main ewe flock during February 1954–May 1956. There were two periods in which the egg counts rose above 200 e.p.g. These were November1954–March, 1955 and October 1955–February 1956. During the rest of the observations the egg counts fluctu-

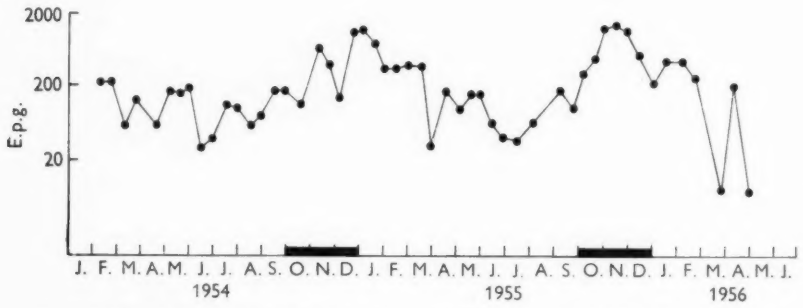


Fig. 1. Flock A. Autumn lambing. Worm egg counts June 1954–May 1956.

ated between less than 10 and 200 e.p.g. In December 1954–January 1955 and November 1955 egg counts of more than 1000 e.p.g. were recorded. The periods of lambing were October 1954–January 1955 and October 1955 to the end of December of the same year. It will be noted that the rises in egg counts occurred some 6 to 8 weeks after lambing in each year.

(*b*) *Flock A lambs, hoggs, and ewes*

Fig. 2 summarizes the egg-count data obtained by sampling the ewe-lambs born in the autumn of 1953. Counts were started in February 1954 and continued until April 1956 when the sheep were then about $2\frac{1}{2}$ years old and when all had lambed once and most had lambed twice.

During the first spring and summer of their lives, the egg counts rose from less than 200 e.p.g. in March to more than 700 e.p.g. in September. By November the egg counts were below 200 and remained low in those which had not lambed. In the sheep which had lambed the egg-counts rose until the mean flock count

reached 600 e.p.g. in January. The egg counts fell again and remained below 200 e.p.g. from March until November 1955. At this time most of the sheep had lambed and, after lambing, the mean egg count rose to 1100 e.p.g. After January 1956 the egg count remained below 200 e.p.g., falling to less than 10 e.p.g. in March.

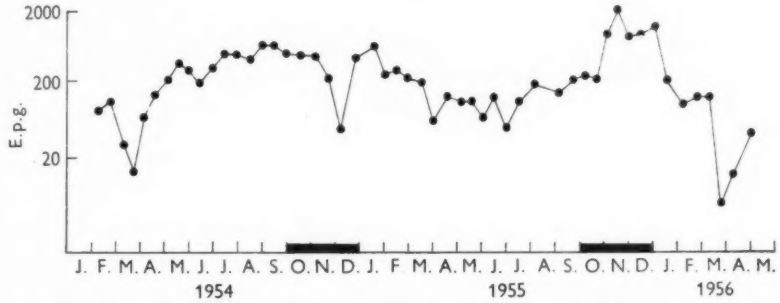


Fig. 2. Flock A. Worm egg counts on sheep born in October 1953. Some of these lambed in the autumn of 1954 and all of them lambed in 1955.

(2) *Spring-lambing flock*

Fig. 3. summarizes the egg-count data obtained from a spring-lambing flock of Clun ewes (flock B) during the period January 1954–June 1956. For most of the period the mean worm egg counts remained below 200 e.p.g., but, as Fig. 3 shows, the counts rose to more than 1000 e.p.g. after each lambing.

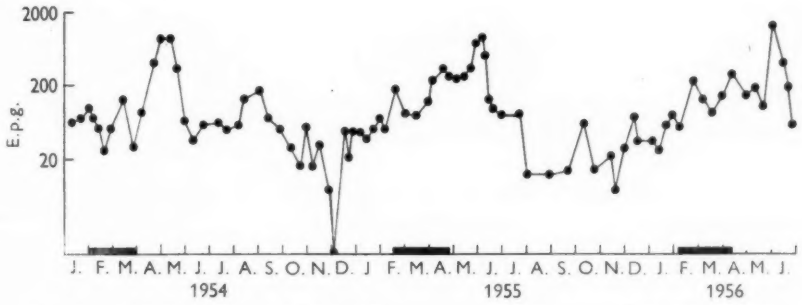


Fig. 3. Flock B. Spring lambing. Worm egg counts January 1954–June 1956.

(3) *Spring- and autumn-lambing flock*

Fig. 4 summarizes the egg-count data obtained from flock C during the period November 1954–December 1956. Because of frequent culling and replacement in the flock, the individuals sampled changed during the period, but mean counts are given throughout. In this flock about half the ewes lambed on each lambing occasion, that is in April–June 1955, November 1955–January 1956, March–May 1956, October to mid-November 1956. Many ewes lambed on successive occasions, and during their short stay in the flock they were either in lamb or lactating.

Again, it will be seen that the egg counts remained low (0–200 e.p.g.), except for short periods after lambing. During May–August 1955, January–March 1956, May–June 1956 and November–December 1956, the egg counts rose to more than 600 e.p.g., on one occasion a mean egg count of 1800 e.p.g. being recorded. The highest individual count was 3300 e.p.g.

Fig. 4. Flock C. Spring and autumn lambing. Worm egg counts November 1954–December 1956.

(4) *Rises in non-reproductive sheep*

Observations made on wethers and barren ewes have shown that in many cases there is an annual rise in egg count during spring. Usually this occurs in April, but in some individuals and in some years it may occur in March, May, or even in early June. In wethers and in young unmated ewes the increase in egg count is rarely strongly defined, there being a gradual increase from less than 200 e.p.g. to a maximum of 700 e.p.g. in individual sheep. The higher egg count persists for about 2 months and then falls gradually to less than 300 e.p.g. in a period of 2 or 3 weeks. Even in autumn-lambing flocks there is no autumn rise in these sheep. In old barren ewes the rise in spring is similar to the post-parturient rise described for ewes with lambs; in these ewes the period of increased counts is usually restricted to less than 1 month, but the increase in individuals is relatively small and the maximum individual counts are never more than 800 e.p.g.

IV. DISCUSSION

The evidence presented supports the view that a rise in egg count in ewes occurs 6 to 8 weeks after parturition, and that this rise is unrelated to the season.

The explanation suggested by Morgan and his co-workers (Morgan *et al.* 1951) cannot apply to the rise in egg counts demonstrated in autumn-lambing flocks and it is clear that the theory of Naerland (1949) is equally unacceptable. It might be suggested, however, that the rise in egg counts in autumn-lambing flocks was not comparable with the so-called 'spring rise'. The autumn rise described in this paper has the same characteristics as the rise in spring-lambing ewes: it is of short duration in individual animals; the maximum count in each individual was always more than 800 e.p.g. and frequently more than 1500 e.p.g.; the rise in individual

sheep occurred six to nine weeks after parturition; no connexion with climatic changes was apparent.

In wethers and in young virgin ewes a spring rise has been recorded from individuals in both spring- and autumn-lambing flocks. The relatively slow increase in egg count, the low maximum and the more gradual reduction suggest that this is a different phenomenon to the rise occurring in ewes which have lambed. It is interesting to note here that ewes which had lambed in autumn but not in spring had an autumnal post-parturient rise but no spring rise. Barren ewes which lambed in neither autumn nor spring sometimes showed a spring rise which was similar to a post-parturient rise in its rate of increase and duration, but of much lower intensity.

It is possible that there are two separate phenomena: in ewes a post-parturient rise to which can be applied the theory propounded in a previous paper (Crofton, 1954); in wethers and possibly in young virgin ewes a spring rise for which the explanation given by Morgan *et al.* (1951) might be acceptable. It is difficult to decide from the data available whether the 'spring rise' of Morgan *et al.* (1950, 1951) is a post-parturient rise or a 'spring rise', because the frequency of sampling is such that no distinction in terms of duration of the period of increase can be made. Examination of the figures suggests that both types of rise in egg count may have occurred.

The occurrence of a rise in egg count in some barren ewes is of considerable interest. In no case did such an increase occur in the autumn. During spring, the increases, although of the same type as those found in ewes with lambs, were smaller and occurred at various times from March to June. The post-parturient rise and the rise in barren ewes are both basically related to the reproductive cycle. Comparison of Dorset Horn flocks with normal spring-lambing breeds shows that there is a greater scatter of times of occurrence of increase in egg count in barren ewes in Dorset Horn flocks than in other breeds. This may be related to the extended breeding season of Dorset Horn sheep and gives support to the suggestion (see Crofton, 1954) that the rise in egg counts is caused by a loss of resistance, which is related to changes in the reproductive cycle, the timing of the post-parturient rise being precise because the time of parturition is itself a precise indicator of the stage of the reproductive cycle.

In a previous paper (Crofton, 1954) it was stated that the high rate of egg production some 6–8 weeks after lambing would provide a nucleus of infective stages available to the young and susceptible lambs. Numerically, the assessment of the importance of the post-parturient rise is not difficult and Figs. 5 and 6 have been constructed to show the total number of worm eggs passed per day by ewes and lambs in spring- and autumn-lambing flocks. The figures are based on worm egg counts carried out on lambs and ewes in two flocks. Flock output has been calculated from estimates of the amount of faeces produced by sheep of different ages. A logarithmic transformation of the raw data was used throughout.

It will be seen from Fig. 5 that in a spring-lambing flock the worm egg output from ewes was approximately one-third of the total output, most of the worm eggs being passed during spring months. The major contribution from the lambs was

in late summer, and it is interesting to note that in the flock considered the rate of contamination of the pasture by lambs did not exceed the spring contamination until August. Thus, it is clear that there were two main periods of heavy contamination of the pasture, one in spring attributable to the post-parturient rise in the ewes and the other in late summer and early autumn associated with heavy infections in lambs.

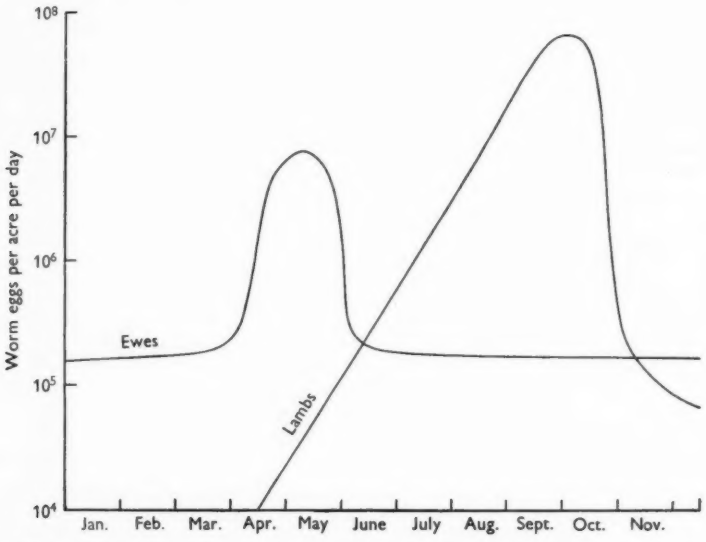


Fig. 5. Flock output of worm eggs expressed as the number of eggs per acre per day, for a flock lambing during spring. (See text for method of estimation.)

Fig. 6 shows the worm egg output of ewes and lambs in an autumn-lambing flock. Comparing this with Fig. 5, it will be seen that the heavy contamination of the pasture by lambs was approximately at the same time as in the spring-lambing flock, although in the case considered the actual number of eggs was lower. The larger number of eggs passed by ewes was mainly concentrated in the post-lambing period in late autumn and early winter.

Thus in both spring- and autumn-lambing flocks there were two main periods of heavy pasture contamination.

In the spring-lambing flock the contribution from the ewes was followed by a logarithmic increase in egg output in lambs. In this respect the rise in egg count of the ewes must be assessed in terms of potential increase in the lambs. In the case of the high output of eggs by lambs in late summer and early autumn the potentiality of each egg must be much lower, because of the lack of susceptible host individuals. Nevertheless, the lamb output must be of great importance to the survival of the parasite population, because the over-wintering populations on the pasture and the relatively small numbers of worms in nonsusceptible animals are the only means of persistence of the infection from year to year. We may regard the two periods of high egg output in such a flock as serving the purpose of

ensuring survival of the parasite population, but each period of heavy contamination contributes in a different way, or has a different emphasis. The post-parturient rise acts as a primer to the main period of multiplication and as such the potential of each egg passed out in faeces is relatively high. The increase in output of lambs is the outcome of this phase of multiplication and is a necessary time-distributive phase in a period when hosts are relatively resistant. The individual eggs at this stage have a relatively low potentiality, but this is counter-balanced by a high population level and an extended time of persistence resulting from a slower rate of development during the winter period.

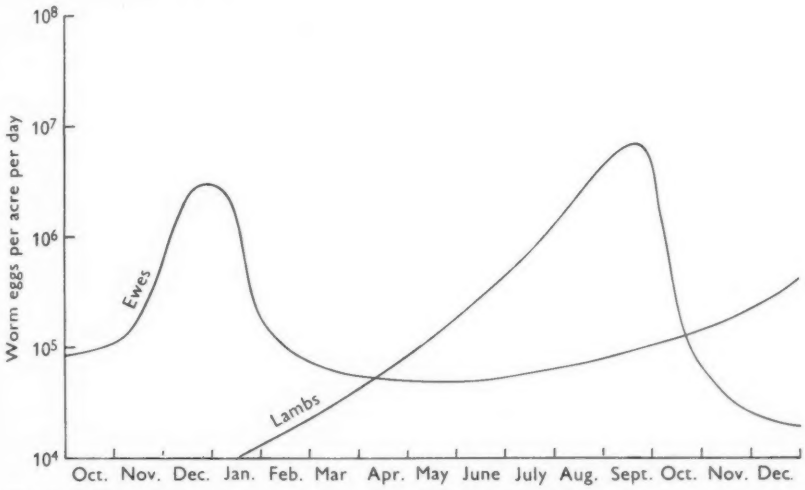


Fig. 6. Flock output of worm eggs expressed as the number of eggs per acre per day for a flock lambing during autumn. (See text for method of estimation.)

In the autumn-lambing flock conditions are somewhat different. The post-parturient rise occurred just before or during the winter, when the rate of development of infective stages was greatly reduced. Thus, although susceptible animals were available, the pressure of infection was low. As will be shown in a later paper, the main infection of lambs does not occur until the following spring and in this respect the time of the multiplication stage is not essentially different to that seen in lambs born 4 months later. In autumn-lambing flocks the high egg output of lambs and the post-parturient rise in egg count both occur in autumn and both contribute to the time distributive phase. The post-parturient rise does not have the same priming effect as in the spring-lambing ewes, and eggs produced by both ewes and lambs in autumn-lambing flocks have the same potentialities. The effect of this on the differential survival of species is being investigated and will be reported later.

The author is indebted to the Agricultural Research Council for financial assistance in the form of a special research grant, and to Mr E. J. Sands for technical assistance.

REFERENCES

CROFTON, H. D. (1954). Nematode parasite populations in sheep on lowland farms. I. Worm egg counts in ewes. *Parasitology*, **44**, 465–77.

MORGAN, D. O., PARNELL, I. W. & RAYSKI, C. (1950). Further observations on the seasonal variation in worm egg output in Scottish hill sheep. *J. Helminth.* **24**, 101–22.

MORGAN, D. O., PARNELL, I. W. & RAYSKI, C. (1951). The seasonal variations in the worm burden of Scottish Hill sheep. *J. Helminth.* **25**, 177–212.

NAERLAND, G. (1949). Nutrition in relation to nematode parasitism in sheep. *XIVth Int. Vet. Congr. London*, 1949, Sect. I*b*, 1–6.

(*MS. received for publication* 15. v. 1957.—Ed.)

NEMATODE PARASITE POPULATIONS IN SHEEP ON LOWLAND FARMS

VI. SHEEP BEHAVIOUR AND NEMATODE INFECTIONS

By H. D. CROFTON

Department of Zoology, University of Bristol

(With 2 Figures in the Text)

CONTENTS

		PAGE
I.	Introduction	251
II.	Distribution of sheep	252
	(*a*) Methods and theoretical approach	252
	(*b*) Results	253
III.	Movements of flocks	255
	(*a*) Direct observation	255
	(*b*) Faecal pellet distribution	255
IV.	Discussion	256
	References	260

I. INTRODUCTION

It is usual, when considering the trichostrongyle infections of sheep, to refer to parasitic stages in the host and free-living stages on pasture as two separate entities with ecological, structural and physiological differences emphasized by their temporal relationship. They are, however, interlinked phases in a continuous exchange process in which the host acts as a vehicle. The role of the host as a mediator in this exchange has received little attention until recently and has been mainly restricted to a more dynamic approach to problems of immunity. The behaviour of the host, as well as that of the parasite, is obviously of great importance, but there has been little work on the behaviour of sheep in relation to patterns of helminth infections, although Taylor (1954) discussed the problem in general terms.

It has been shown (Crofton, 1952) that over a small area selected from a pasture the distribution of infective larvae could be described by one of Neyman's contagious distributions. This was explained in terms of the infective larvae moving only small distances laterally from clumps of faecal pellets which, within the areas selected for sampling, were distributed in an apparently random manner. Although this applied to selected areas of pasture, it is obvious from inspection that clumps of faeces are not distributed randomly over a pasture as a whole.

Hunter & Quenouille (1952) have shown that within a series of samples of faeces from a flock of sheep the distribution of egg counts approximates to a Negative Binomial distribution. The relationship between the number of parasitic stages in the sheep and free-living stages on the pasture must, therefore, be a complicated one, even if the sheep grazed and distributed their faeces at random over the whole field.

Preliminary observations made on the distribution of faecal pellets and grazing sheep suggested that neither of these was random, and more detailed work is described here.

II. DISTRIBUTION OF SHEEP

(*a*) *Methods and theoretical approach*

After a preliminary survey of several flocks from the ground and from various buildings and other points of vantage, it was decided that the only satisfactory method of studying sheep distribution would be by means of aerial photographs. Photographs of fields were obtained from survey pictures taken for another purpose, these being particularly suitable because all the necessary data were available for the calculation of the size of the fields and their precise location. Most of these photographs were of different fields, but one group was obtained which gives a series of photographs of the same field at different times.

The analysis of these photographs presented some difficulty. Although examination suggested some type of aggregation, a departure from randomness could not be assumed.

There are various theoretical methods of testing for random scatter in two dimensions. The most obvious one is to measure the distance of each sheep from every other sheep and to plot the distribution of numbers at different distances and at the squares of the distances. Such a method is laborious, but the labour can be reduced by using an annular grid. From the results it is theoretically possible to determine how the sheep are distributed; by simple computation even spacing and random distribution can be distinguished easily. In practice, however, the method cannot be applied unless the population is very large, and with small flocks, none of them consisting of more than eighty animals, no reliance can be placed on the results given by this method.

A second method was suggested during discussions with Professor F. C. Frank. This is dependent upon the theorem that, if particles are distributed over a surface, then points which are equidistant between neighbouring particles are arranged in a polyhedral pattern. If the number of points is infinite, or, if they are arranged on a sphere, then the mean number of sides of the polyhedra will be six. If the particles are evenly spaced, all the polyhedra will be regular hexagons equal in area; departure from even spacing will be reflected by the variation in area, regularity and number of sides of the polyhedra. While this applies to an infinite system, a correction may be applied if the number of particles is finite.

The problem can be approached in a way more applicable to a finite pattern.

If organisms are evenly spaced, and if we select any one, the angles subtended by any other two will fall within a discontinuous series, the intervals being multiples of $120°/n$, where n is a function of N, the total number of organisms present. If measurements are restricted to adjacent organisms, then the angles will be 60°, 120° and 180°. This provides a method for testing for even spacing and, arising from it, a method of testing for random scatter. For random distributions the angles measured would fall in a continuous series. Obviously the method is very

laborious if the population considered is large, and with other than small groups a sampling method must be applied.

All three of the methods outlined were applied to the data.

(*b*) *Results*

Measurements of the distances of each sheep from every other sheep in the flock did not show any evidence of even spacing of the sheep. Because of the relatively small flocks considered, the data for any one flock were inconclusive, but highly suggestive of a considerable departure from a random distribution. Comparison of measurements for different flocks gave evidence of another nature, of interest in itself, and also indicative of a non-random scatter of individuals. In more than 90% of the cases considered the mean distance between one sheep and the others in a flock was between 15–30 yards, even in fields of different sizes (12–55 acres). While the mean distance between sheep varied from pasture to pasture, there was no relationship between the mean distance and the size of the field, the number in the flock or the number of sheep to the acre.

The plotting of the neighbour space results in different flocks producing a variety of polyhedra of greatly different sizes and numbers of sides. The most frequently occurring polyhedra were hexagons, but these were very irregular and differed considerably in area. The only conclusion that can be drawn from this is that the sheep were not evenly spaced.

Measurement of subtended angles produced an unexpected result. Although the angles measured did not produce a markedly discontinuous series, the angle 110° occurred more frequently than any other. To test whether or not this was merely a random departure from 120° the relevant angles were remeasured. The mean of the new measurements was $111{\cdot}9° \pm 3{\cdot}6°$. It is reasonable to assume that the angle which occurred most frequently was not connected with even spacing—a conclusion supported by the previous analyses.

Detailed re-examination of the aerial photographs showed that the majority of sheep were orientated so that two other sheep subtended an angle of approximately 110° to it. This occurred when the two sheep were in front of the individual and never when they were behind it, suggesting that a visual method of orientation might be involved (Fig. 1). There appeared to be no constancy in the distance between the sheep so orientated, although the maximum distance between the individual and its reference sheep was approximately 30 yards, the average distance being approximately 15 yards.

In no flock were all the sheep so orientated. At first it was assumed that, if there were constant adjustment in the flock during grazing, then photographs taken during adjustment would fail to show a fixed angle phenomenon for all the flock. Observation in the field suggested three other reasons for the exceptional cases.

First, the maintenance of a constant angle did not apply to sheep which were not actively grazing. Secondly, it was apparent that a sheep might have only one other sheep in view under certain circumstances. The most usual example of this was when a sheep was grazing near the boundary of a field. In this case there always appeared to be a prominent landmark which might be substituted for one

reference sheep. It was observed that in many of these cases the sheep at the edge of the field tended to rotate as it grazed when other sheep moved, the observer, a tree or some other sharply defined object being the fixed reference point. Further examination of the aerial photographs showed that some anomalous cases could be explained on the basis of prominent landmarks in or around the field providing fixed points of reference for the sheep. The third reason was not deducible from aerial photographs, but was apparent from field observation. In some cases all the sheep appeared to be orientated in approximately the same direction. Under

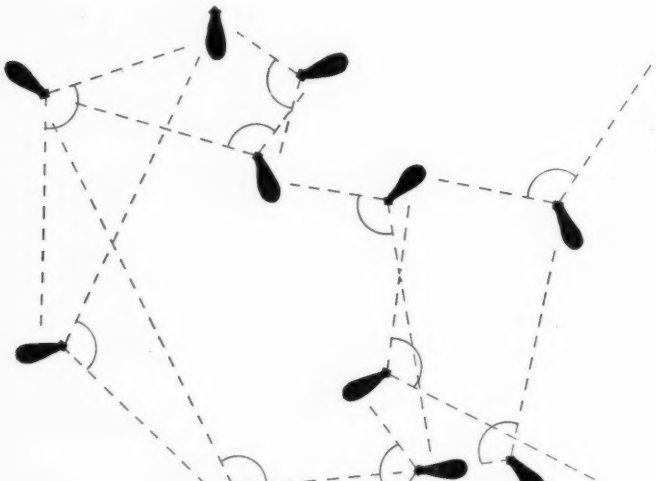


Fig. 1. Diagram of the distribution of grazing sheep in a portion of a flock. The angles marked are all approximately 110°.

these conditions most of the flock could maintain position by visual reference during grazing, but the leading sheep were obviously in no position to do so. The leading sheep were observed to graze less continuously than the others and frequently raised their heads. Flock behaviour of this type was observed only when there was a relatively strong wind or wind and rain.

All the above results were obtained with mature sheep, but some observations were made on lambs and hoggs.

In the case of ewes with lambs there was considerable departure from the basic pattern described and, although the ewes tended to keep two other sheep as points of reference, grazing was interrupted more frequently by the head being raised. As the lambs became older there was greater conformity to the pattern, the periods of continuous grazing becoming longer. The lambs, however, grazed spasmodically and appeared to relate their position to another sheep—not always their dam.

After weaning, the ewes reverted to the pattern already described, but the hoggs

grazed in fairly active bands in which the individuals were more closely packed than in the ewe flock. No evidence of orientation by angle was obtained and the general behaviour suggested that position in the flock was maintained by the individuals remaining fairly close to each other. There was a greater tendency for temporary subflocks to form, but these were relatively quickly reabsorbed into the main group.

Ewe flocks occasionally split into two, but rarely into three, subflocks. This subflocking occurred in the larger units and could be maintained for several hours before reunion occurred.

III. MOVEMENTS OF FLOCKS

(*a*) *Direct observation*

Observations were made on flocks of sheep in various pastures and the areas grazed at different times was noted. No night observations were made because of technical difficulties, but from the observations made on faecal pellets it seems probable that observations during darkness might have been of considerable significance.

On pastures which had been grazed for several days the ewe flocks appeared to have less tendency to subdivide than on pastures to which they had been recently introduced. In a newly stocked pasture there tended to be considerable movement of the flock as a whole and considerable areas of the pasture were 'raked'. After a few days the movements of the flocks were more restricted and after 4 or 5 days there appeared to be a greater time spent in certain parts of the field than in others. Visually no great difference in the herbage could be detected. After a few more days there appeared to be some pattern of flock movement in the field, but its nature could not be analysed. There was an apparent avoidance of grazing of some areas, but no differences in the herbage could be distinguished. The areas not grazed were not deficient in quantity of herbage. Later it was noticed that areas previously avoided were grazed without there having been time for any appreciable growth.

The significance of these observations was only understood when the work described in the next section was completed.

(*b*) *Faecal pellet distribution*

Observations were made on the distribution of faecal pellets, using the sampling method described in a previous paper (Crofton, 1952).

In a newly stocked field there was at first no marked pattern of distribution of faecal pellets, but after a few days there were areas with a much higher concentration of droppings than in other parts. The areas of high concentration did not correspond with areas which had been particularly heavily grazed and it became apparent that the highest concentrations must have been produced during the hours of darkness. Once these areas had been recorded it was clear that the flock as a whole tended to avoid them during grazing, but that the avoidance was not permanent because grazing of a heavily contaminated area might occur 5–8 days

after the concentration had been first noted. By this time there would be several other areas with concentrations of faecal pellets at least as high as the first. These new areas were not grazed and there tended to be no great increase in contamination of an area.

It was apparent that faecal pellets were not distributed randomly and that areas of high concentration appeared in different parts of the pasture. These areas did not necessarily correspond to the most heavily grazed areas of the past, but they were avoided for several days.

IV. DISCUSSION

It is clear from the above observations that sheep do not graze randomly over a pasture. The individuals aggregate to form a flock and this aggregation is related to the orientation of the individuals during grazing. The observation that each grazing sheep tends to maintain its position by reference to two other sheep situated in front of it and forming an angle of 110° is of considerable interest. It is

Fig. 2. Diagram to show the angle between the optic axes in a grazing sheep. The angle is 110°.

apparent that, as the two points of reference must always be in front of the sheep, the fixed angle is most likely to be maintained visually. The angle corresponds to the angle between the optic axes of the eyes and as such it could be maintained without any special adaptation (Fig. 2). The value of this to the sheep is obvious. In an animal which under natural conditions tends to live in a two-dimensional world, the maintenance of position in relation to others of its kind will be most easily achieved by two reference points. The restricted field of binocular vision permits some appreciation of distance when the animal is not grazing, but with its head down the field of binocular vision is restricted to an area of ground in front of it. Because of the necessity for prolonged grazing, an orientation device independent of the head being raised is of great advantage. Because the head is

maintained close to the ground, the effective distance of this device is limited, but is sufficient to permit some degree of freedom of movement of the individual without loss of contact with the other members of the flock. Such a device has another advantage. If position is maintained visually as described, then slight movements in the flock will require only slight adjustment of the individual animals. A small disturbance at one side of the flock will be unlikely to cause any need for readjustment at the other side. A large disturbance, however, will be transmitted to all individuals. This is of considerable biological importance to an animal which must spend most of its life grazing, but must still be warned of the approach of predators.

Young lambs do not show this type of orientation, but because of their close association with ewes they are incorporated into the main flock pattern. Fixation of position by reference to individual ewes will require more frequent use of binocular vision and this will be achieved only by reduction in grazing time—a matter of small importance in a suckling animal.

Hoggs, usually seen in small bands, appear to show a transition between lambs and ewes in their method of flock formation. The relatively close proximity of individuals is similar to the arrangement of lambs in relation to ewes. Increased grazing time can be achieved by the ewe method of orientation, but the more compact flock will tend to result in a greater activity of the flock as a whole, because small movements will produce greater angular displacements. This greater activity is characteristic of hoggs. The necessity for more prolonged grazing will tend to produce a greater dispersion of individuals and a gradual adoption of the ewe flock pattern.

It must be realized that separation of hoggs from ewes is to some extent an artificial product of domestication and the transition from the suckling lamb to hogg is likely to be more gradual in a wild flock. During this transition the fixed pattern of the ewes will stabilize the flock.

The fixed angle of 110° is of some interest in the composition of flocks and the tendency to form subflocks. Obviously the minimum number in a flock for the conditions to be satisfied is five, although the minimum number will give a small degree of flexibility. An increase in the number can be accommodated in an increasing number of ways. In theory, with ten individuals there is the possibility of two subflocks being formed, but the chances of such a split are comparatively low. With greater numbers the chances of five or more sheep being independent of the main flock will be increased, but as the number of sub-flocks increases in a restricted area the chances of union of two subflocks will also increase. Thus for a finite pasture there will be a dynamic equilibrium which will determine the level of subflock formation.

The relevance of this to trichostrongyle infections is apparent when the distribution of faecal pellets is considered.

The flock habit results in non-random distribution of faeces over the pasture. The relatively high concentration of pellets in parts of the pasture may be explained in two main ways: (1) incomplete utilization of the pasture by the flock, (2) a synchronization of defaecation within the flock.

In the case of a flock placed on a 'clean' pasture there appears to be no definite pattern of flock movement, but the aggregation of individuals is unlikely to produce even grazing over the pasture except over very long periods. From the observations described above there appears to be some avoidance of areas already contaminated. While further work is necessary on this point, it is clear that avoidance of some parts of the pasture is likely to increase contamination in other parts, so that after a period it is likely that the pasture utilization will be fixed according to the previous history of the grazing and contamination.

Synchronization of defaecation seems at first to be unlikely, but defaecation is not a continuous process and must be regulated to some extent by the period of ingestion and the rate of the digestive processes. While grazing necessarily occupies a large portion of each day's activities, and, although different individuals may have different periods for ruminating and resting, there will be imposed a rhythm of defaecation if all the flock are prevented from grazing for the same period of time. From general observations made there appears to be at least a great reduction in grazing throughout the hours of darkness; and this in itself, by establishing a pattern of intake, is likely to produce some degree of synchronization of defaecation in the flock. Examination of pasture suggests that reduced movement of the flock at night produces areas of high faecal contamination. Whether or not this contamination is more pronounced because of a higher rate of defaecation at night than during day has not been established.

The avoidance of heavily contaminated areas is still being investigated, but its parasitological importance is considerable. The duration of avoidance has been observed to vary from 5 to 8 days and, while more detailed observations have still to be made, it must be noted that during this period the developmental stages of most trichostrongyle parasites in the faeces will be non-infective. Only after a week or more will infective larvae be available. Thus the avoidance of fresh faecal contamination will effectively reduce the area of pasture grazed, the reduction being mainly due to a loss of an area of pasture with few infective stages but of high parasite potential. The regrazing of the area will tend to coincide with the maximum numbers of infective larvae.

There has always been a tendency to regard avoidance of faecal contamination as a system of hygiene of selective advantage to the host animal, but it must be remembered that for the persistence of the host-parasite relationship there must be opportunity for infection. The type of synchronization described is one which would be of selective advantage favouring the specific relationship between the sheep and its trichostrongyle parasites. The supposition that avoidance of faecal contamination is part of a pattern of hygienic measures adopted by a host animal to limit infection is based upon the assumption that parasites are harmful to the host species. While it is undisputed that parasitic infections damage and even kill individuals, this does not necessarily mean that parasitic infection does not favour the survival of the host species under natural conditions.

Man, as a domesticator, however, has no interest in the survival of the host species in general, but he has a very direct interest in the survival of individuals or groups of individuals. The relationship between host and parasite is one which he

would prefer to end and he would be content to regulate the number of his animals in his own way without the intervention of the parasite. Without the elimination of parasites he must attempt to produce an apparently contradictory state of affairs—a reduced chance of parasitic infection and a high density of domestic stock. He can control numbers by regulating breeding and controlling slaughtering. The provision of adequate food supplies involves a system of management which will ensure efficient utilization of available pasture without increasing parasitic infection. The basis of any such system must be a carefully planned stocking policy.

The rate of stocking of sheep on pastures has been generally recognized as an important factor in sheep parasite control. The automatic regulation of grazing by temporary avoidance of contaminated areas complicates the position, but the pattern of flocking behaviour in itself demonstrates the futility of discussing stocking rates in terms of sheep per acre. It has been shown that the effective size of the flock as measured by the mean distance between sheep bears no direct relationship to the number of sheep in the flock, the size of the field or to the number of sheep per acre. The greater tendency to form subflocks as the number of sheep increases adds to the difficulty of interpretation.

There are, however, a number of practical considerations which must be remembered. The total amount of pasture contamination must bear a direct relationship to the number of sheep grazing, but the *concentration* of faeces in local areas of the pasture is not necessarily a direct function of the number of sheep. The chances of return to a previously contaminated area will be related to the available amount of herbage per sheep and to the time spent in a particular pasture. Thus a low stocking rate in the conventional sense is likely to reduce the chances of infection, but this reduction in infection must be balanced against efficient pasture utilization. Because of the flocking pattern an effective low stocking rate may be achieved by stocking a pasture with a high proportion of sheep to the acre for a short period, this period being less than the interval between the time of contamination of an area and the time of return of the sheep to graze it. This is, in fact, the general rule which has been long propounded.

In practice, however, a low stocking rate, whether it be achieved by running small numbers of sheep per acre or by short periods of heavy grazing, is unlikely to give the most efficient pasture utilization. Intermediate stocking levels are usually seen in commercial flocks and it is in these cases that difficulties of interpretation arise. The present work suggests that the effect of change in actual stocking rate at these intermediate levels is not likely to have a directly proportional effect on the chances of infection, unless the period of occupation of the pasture is relatively long.

The author is very grateful to Mr G. W. Jones, of Aeroplane and Armament Experimental Establishment, for the help and advice in overcoming many of the technical difficulties in this work; to Mr R. Pleasants, also of A. and A. E. E., for his advice and help with special photographic techniques, to Professor F. C.

Frank for advice on distributions and to Mr E. J. Sands for technical assistance. Financial assistance in the form of a special research grant was provided by the Agricultural Research Council.

REFERENCES

CROFTON, H. D. (1952). The ecology of immature phases of trichostrongyle nematodes. IV. Larval populations on lowland pastures. *Parasitology*, **42**, 77–84.

CROFTON, H. D. (1954). Nematode parasite populations in sheep on lowland farms. I. Worm egg counts in ewes. *Parasitology*, **44**, 465–77.

HUNTER, G. C. & QUENOUILLE, M. H. (1952). A statistical examination of the worm egg count sampling technique for sheep. *J. Helminth.* **26**, 157–70.

TAYLOR, E. L. (1954). Grazing behaviour and helminthic disease. *Brit. J. anim. Behav.* **2**, 61–2.

(*MS. received for publication* 22. v. 1957.—Ed.)

THE EFFECTS OF TREMATODE METACERCARIAE (BRACHYLAEMIDAE) ON THE SLUGS *MILAX SOWERBII* FÉRUSSAC AND *AGRIOLIMAX RETICULATUS* MÜLLER

By R. FOSTER, B.Sc., Ph.D.

*Zoology Department, The Durham Colleges in the University of Durham**

(With Plate X)

CONTENTS

		PAGE
I.	Introduction	261
II.	Histological effects	262
III.	The lethal effect	262
IV.	*Agriolimax reticulatus*: the effect on feeding	265
V.	*Agriolimax reticulatus*: the effect on fecundity	265
VI.	Discussion	266
VII.	Summary	267
	References	268

I. INTRODUCTION

Unlike the effects of adult flukes on vertebrate hosts, the effects of trematode larvae on intermediate hosts are rarely of medical or veterinary significance and have received comparatively little attention. Moreover, most previous work has been confined to the effects of the redial, sporocyst and cercarial stages (Faust, 1920; Agersborg, 1924; Hurst, 1927; Wesenberg-Lund, 1931, 1934; Rees, 1936; Rothschild, 1936, 1939, 1941). Brown (1926) and Hurst (1927) have stated that the encysted metacercariae have no effect upon the host, but a number of authors (particularly Krull, 1935; Balozet, 1937; Dollfus, 1938, and Ulmer, 1951) have noted that during the earlier and more active phases the intestines of metacercariae become filled with substances resembling the tissues which the parasites inhabit.

The metacercariae of some species of the family Brachylaemidae remain unencysted, and those of other species possess only a thin, jelly-like membrane, which surrounds occasional individuals, and which is not regarded as a true cyst (Ulmer, 1951). The finding of completely unencysted brachylaemid metacercariae by Cragg, Foster & Vincent (1957) made it possible to investigate whether these metacercariae may have any effects on the lives and activities of their hosts.

The hosts of the metacercariae investigated were the slugs *Milax sowerbii* Férussac and *Agriolimax reticulatus* Müller. Colonies of both these species were maintained in the laboratory. They consisted of wild slugs collected from localities known to be infested with brachylaemids. The degree of infestation of each colony of slugs was determined by dissection of the slugs which died and of the survivors

* Now with the Colonial Research Service, Tanganyika.

at the close of the observations. By collecting from different localities it was possible to establish a series of colonies covering the range of 0–100 % infestation.

The metacercariae were found to exert profound pathological effects on their hosts.

II. HISTOLOGICAL EFFECTS

In *A. reticulatus* the kidney is a small, almost spherical body, about 2 mm. in diameter. It is ochreous-yellow in colour and lies immediately beneath the mantle cavity, slightly posterior and dorsal to the pericardium. In *M. sowerbii* it is about 4–5 mm. in diameter, flattened dorso-ventrally, and encircling the pericardium. In both species it is enclosed by a thin membrane and communicates with the pericardium via a renopericardial canal and with the exterior via a thin-walled ureter.

Histologically the kidney is of a connective and vascular nature, with the lumen completely filled by a mass of folds and lamellae. The main mass of the organ is composed of well-defined relatively thick-walled cells. The main part of each cell is occupied by a vacuole and the nucleus, if it appears at all, does so as a darkly staining mass close to the lamellar border of the cell. Many of the cells contain granular inclusions and the whole organ is permeated by a plexus of blood vessels. The structure of the healthy kidney of *A. reticulatus* is shown in Pl. X, fig. 1.

The metacercariae, which inhabit only the kidney of the host, have four stages in their life history successively designated caudal, feeding, corrugated and smooth (Cragg *et al.* 1957). Infestation by caudal-stage larvae produced no evident histological changes, kidneys supporting up to 150 of these parasites exhibiting a normal appearance. When, however, these young forms changed into feeding-stage larvae, extensive anatomical changes in the host tissues were observed. A general necrosis originated in the area immediately surrounding the parasites (Pl. X, fig. 2) and quickly spread throughout the whole organ, even if the parasites inhabited only a part of it. The lamellae disintegrated and disappeared and the cells became isolated from each other and heavily laden with concretions. Later the cell walls broke down and the kidney became a mass of necrotic cell debris, isolated remnants of lamellae, and granular masses (Pl. X, fig. 3). Eventually much of this mass disappeared, leaving only the connective tissue shell of the kidney surrounding the parasites and scattered patches of detritus (Pl. X, fig. 4).

The dense mass which fills the gut of the feeding-stage metacercariae (Cragg *et al.* 1957) consists of necrotic kidney tissue and its inclusions.

When the kidney was disorganized to the extent shown in Pl. X, fig. 3, the metacercariae were usually in the later phases of the corrugated stage, although sometimes a few feeding-stage larvae were still present. The smooth-stage larvae were found lying freely in clear spaces among the scattered patches of detritus.

The histological effects were similar in both host species.

III. THE LETHAL EFFECT

Slugs collected from various localities during the spring and summer months were maintained in colonies, and the effect of parasitism on their longevity was investigated. Although the colonies were maintained for up to 6 months, the data given

here are derived from the 25-day period after each colony was established; this allows comparisons to be made between colonies and reduces the chances of including deaths from old age.

(*a*) *Effect on* Agriolimax reticulatus

The parasitization of this species led to a degree of mortality among the hosts which apparently equalled the incidence of infestation.

Table 1 shows the correlation between the incidence of parasitization and host mortality. It is seen that colonies 12 and 13, which were free from parasites, showed a combined mortality of only 5%, whereas colonies 1–6, all of which had infestations of over 80%, showed mortalities of 78% or higher.

The connexion between mortality and infestation is shown in Table 2. In

Table 1. Agriolimax reticulatus: *incidence of infestation and mortality (25-day period)*

Colony	No. of slugs	Percentage infestation	Percentage mortality
1	11	100	100
2	10	100	90
3	10	90	80
4	10	90	100
5	37	89	89
6	18	83	78
7	10	60	90
8	10	40	20
9	13	39	69
10	9	22	56
11	10	20	10
12	10	0	0
13	10	0	10

Table 2. Agriolimax reticulatus: *incidence of infestation among dead and survivors*

Colony	Percentage infestation among	
	Dead	Survivors
1	100	No survivors
2	100	(100)
3	88	(100)
4	90	No survivors
5	93	25
6	86	0
7	67	0
8	50	37
9	56	0
10	40	0
11	100	11
12	No deaths	0
13	0	0

colonies 1–6 and 11, over 80 % of the slugs which died were harbouring parasites and, apart from the healthy colony 13, the minimum incidence in any colony was 40 %. But all the survivors of six of the colonies were healthy and, excluding colonies 2 and 3, the maximum incidence of infestation in any batch of survivors was only 37 %. The survivors of colonies 2 and 3 appear to be in contrast to the others, but this is true only for the 25 days covered by the data. Later, the three survivors of these two colonies died and the results then showed 'no survivors', instead of the 100 % infestation among survivors as given in Table 2.

Of the total number (112) of slugs which died during the period, 85 % harboured parasites, but, of the total (56) which survived, only 25 % were infested and some of these died at later dates.

(*b*) *Effect on* Milax sowerbii

M. sowerbii were collected during the spring and summer and maintained similarly to the *A. reticulatus*. Mortality data are given in Table 3.

It is seen that during the summer months the incidence of mortality was not correlated with the incidence of parasitization.

Table 3. Milax sowerbii: *incidences of infestation and mortality (25-day period)*

Colony	No. of slugs	Percentage infestation	Percentage mortality
1	28	75	4
2	15	73	13
3	10	60	20
4	10	50	10
5	20	0	10

Infested colonies were maintained throughout the summer and autumn. The number of slugs remained fairly constant during the summer and early autumn, despite the high levels of infestation, but showed a decline to extinction during the late autumn and early winter (Table 4). Colonies of healthy *M. sowerbii* have been maintained for a number of years with no sign of this high winter mortality.

Although the kidneys of both host species are destroyed by the parasites,

Table 4. Milax sowerbii: *longevity of colony* 2 (73 % *infestation*)

Date	No. of survivors
15 June	15
30 June	15
15 July	14
31 July	13
15 Aug.	13
31 Aug.	12
15 Sept.	12
30 Sept.	12
15 Oct.	10
31 Oct.	3
6 Nov.	0

M. sowerbii appears to survive until the onset of winter. It is suggested that this species is a natural intermediate host of the parasite and that *A. reticulatus* is not (see discussion).

IV. *AGRIOLIMAX RETICULATUS*: THE EFFECT ON FEEDING

Colonies were maintained to which a weighed amount of food (dry lettuce leaf) was introduced daily, and the remains of this food were re-weighed after 24 hr. Observations in a 'control colony' without animals showed that the mean loss in weight of the food due to causes other than ingestion (e.g. evaporation of constituent water) was 1·5 % of the initial weight in 24 hr., with a maximum of 2·4 %.

Table 5. Agriolimax reticulatus: *mean consumption of food (g. per 24 hr.)*

	Slugs 2–4 g. in weight		Slugs 4–6 g. in weight
Observation	Colony 3, 20 % infestation	Colony 5, 90 % infestation	Colony 6, 100 % infestation
1	0·275	0·144	0·193
2	—	0·130	0·193
3	0·200	0·084	0·243
4	0·161	0·106	0·144
5	0·192	0·132	0·114
6	0·279	0·077	0·156
7	0·199	0·083	0·094
8	0·209	0·032	0·127
9	0·165	0·064	0·068
10	0·190	0·025	0·025
11	0·170	0·120	0·100
12	0·195	0·083	0·046
13	0·170	0·073	0·032
14	0·142	0·083	0·042
15	0·064	0·110	0·027

Table 5 shows that the amount of food ingested was reduced in those colonies with a high incidence of infestation. Only once did the slugs of colony 5 (90 % infestation) consume as much food as did individuals of similar size of colony 3 (20 % infestation). Furthermore, only once did the mean consumption of hosts of 4–6 g. weight in colony 6 (100 % infestation) equal that of the smaller slugs in colony 3 (20 % infestation).

V. *AGRIOLIMAX RETICULATUS*: THE EFFECT ON FECUNDITY

Eggs were collected daily from the colonies, and data for fecundity are given in Table 6. The data cover the 25-day period following the establishment of each colony.

It is seen that, excluding colony 8, the mean number of eggs/day/slug was reduced in those colonies supporting higher incidences of infestation.

Attempts to determine the viability of eggs laid by healthy and parasitized *A. reticulatus* were hindered by the inability to devise any technique for hatching slug eggs in a satisfactorily constant manner. The incidence of hatching was low and irregular, and apparently unrelated to the parasitization of the parents.

Table 6. Agriolimax reticulatus: *fecundity and infestation (25-day period)*

Colony	Mean no. of eggs/day/slug	Percentage infestation
1	0·150	100
2	0·420	90
3	0·700	90
4	0·850	60
5	0·640	38
6	1·550	20
7	2·390	22
8	1·640	100

VI. DISCUSSION

Apart from the kidney, all organs exhibited a normal histological appearance, and the colour and activity of the hosts remained normal. The destruction of the renal organ and the reduced intake of food during parasitization may, however, affect the water relations, weight, and growth rate of the host. But healthy slugs do not exhibit a constancy of weight or of hydration of the tissues (Howes & Wells, 1934), and a clearer understanding of the normal state is needed before possible abnormalities can be investigated.

In data to be published later, it will be shown that *Agriolimax reticulatus* becomes parasitized primarily in the spring, the incidence of infestation within a population rising rapidly to a peak in June or July, and then falling steadily throughout the summer and autumn. *Milax sowerbii* also becomes parasitized in the spring, but the incidence of infestation, after reaching the peak in early summer, remains consistently high until a sudden drop occurs in November or December. This field evidence accords with the laboratory finding that *M. sowerbii* tolerates the parasitization better than *A. reticulatus*, and that the death of a host of the former species is considerably delayed in comparison with that of the latter species. Thus *M. sowerbii* is considered to be a natural host of the parasite and *A. reticulatus* to be an unnatural host.

It is thought that the vertebrate host of this brachylaemid is a small mammal. Apparently the metacercariae inflict fatal injuries on both species of intermediate host, but only in *A. reticulatus* does death occur before September, when most of the metacercariae reach maturity. In the natural host death is delayed until the larvae have reached the infestive stage and the vertebrate has been exposed to infestation for some time. By November or December many of the prospective vertebrate hosts are inactive or hibernating and deaths of intermediate hosts during the winter are therefore immaterial.

Although caudal-stage larvae do not produce any observable effects on the host kidney, some of the *A. reticulatus* which died were infested only with this stage. In many cases 100–150 parasites were present and death could have been due to pressure on the heart.

The number of parasites decreases as one metacercarial developmental stage succeeds another, decreasing from about seventy caudal-stage larvae per host to about six smooth-stage forms. To propose a migration out of the host is impracti-

cal, as it implies a desertion of the host by immature larvae which can only attain maturity by remaining in the host. A high mortality within the host also appears to be unlikely, because dead parasites were not found during the dissection of almost 3000 hosts, and dead metacercariae can remain recognizable when they are maintained in kidney tissue for 2–3 days after death. It is possible that this apparent fall in the incidence of infestation arises from the death of those hosts which carry heavy infestations.

The ability of *M. sowerbii* to survive for some months while it is apparently deprived of its kidney function raises several problems, the chief of which is the mechanism whereby survival is effected and the causes of the ultimate death. If waste products are not expelled by an alternative mechanism (e.g. defaecation), they may accumulate at some point in the body and cause the eventual death of the host when they reach a toxic concentration. The different degrees of toleration shown by the two host species may indicate specific physiological differences.

If other species of unencysted metacercariae exert effects on their hosts similar to those described here, it may be possible to use such parasites for the control of certain pests (e.g. *A. reticulatus*). The role of larval trematodes in controlling host populations has been noted previously (Cort, Olivier & McMullen, 1941), but only in connexion with sporocyst and redial infestations. These are economically insignificant owing to the low incidence of infestation by such larvae (about 1 % of the host population for known brachylaemid sporocysts). It is, however, now known that unencysted metacercariae may also exert a lethal effect on their hosts, and in the present study up to 97 % of the host population was parasitized by such larvae. If a suitable vertebrate host were artificially infested and released, a lethal metacercarial infestation may be spread quickly and easily among the molluscan hosts.

A number of difficulties may arise. The greatest effects would probably arise from parasites infesting unnatural hosts. Further, the possibilities attendant on interfering with the 'balance of nature' must be considered. Finally, life histories must be carefully 'fitted'. In the present case, the slugs are naturally exposed to infestation during April and May, when many of them are small enough to be apparently immune to parasitization (as will be reported in a later paper), so most of the host population is therefore healthy during the summer, when it becomes a major pest. With an artificial infestation it may be possible to delay the release of the vertebrate host until such time as the majority of the slug population will have become infested.

VII. SUMMARY

1. Infestation of the kidney of *M. sowerbii* and *A. reticulatus* by unencysted brachylaemid metacercariae led to a widespread necrosis of the organ. The necrotic debris was ingested into the gut crura of the parasites.

2. *M. sowerbii* is considered to be a natural intermediate host of the parasite and *A. reticulatus* to be an unnatural host, because in *A. reticulatus* parasitization led to a host mortality closely related to the degree of infestation, whereas *M. sowerbii* withstood the damaging effects of parasitization until the parasites reached maturity.

3. In *A. reticulatus* the rate of ingestion of food and the rate of egg production were reduced during parasitization.

4. No further effects were observed in either host species.

5. The possibilities and difficulties of using unencysted metacercariae to control pests are discussed.

The author wishes to express his gratitude to Professor J. B. Cragg for valuable advice during the investigation, and for help in the preparation of this paper. The work was financed by the Agricultural Research Council.

REFERENCES

AGERSBORG, H. P. K. (1924). Studies on the effect of parasitism on tissues. 1. With special reference to certain Gastropod molluscs. *Quart. J. Micr. Soc.* **68**, 361–401.

BALOZET, L. (1937). Cycle évolutif de *Brachylaemus suis*. *C.R. Acad. Sci., Paris*, **204**, 622–4.

BROWN, F. J. (1926). Some British freshwater larval trematodes, with their life histories. *Parasitology*, **18**, 21–34.

CORT, W. W., OLIVIER, L. & MCMULLEN, D. B. (1941). Larval trematode infections in juvenile and adult *Physa parkeri*. *J. Parasit.* **27**, 123–241.

CRAGG, J. B., FOSTER, R. & VINCENT, M. (1957). Larval trematodes from the slugs *Milax sowerbii*, *Agriolimax reticulatus* and *Arion lusitanicus*. *Parasitology*, **47**, 396–404.

DOLLFUS, R. PH. (1938). Métacercaire de *Brachylaemus* chez *Helicella obvia* en Bulgarie. *Ann. Parasit. hum. comp.* **16**, 28–32.

FAUST, E. C. (1920). Pathological changes in Gastropod liver produced by fluke infection. *Johns Hopk. Hosp. Bull.* no. 31, 79–84.

HOWES, N. H. & WELLS, G. P. (1934). Water relations of snails and slugs. 2. Weight rhythms in *Arion ater* and *Limax flavus*. *J. Exp. Biol.* **11**, 344–51.

HURST, C. T. (1927). Structural and functional changes produced in the Gastropod mollusc *Physa occidentalis* in the case of parasitization by larvae of *Echinostomum revolutum*. *Univ. Calif. Publ. Zool.* **29**, 321–404.

KRULL, W. H. (1935). Studies on the life history of *Panopistus pricei*. *Parasitology*, **27**, 93–100.

REES, W. J. (1936). Effect of parasitization by larval trematodes on the tissues of *L. littorea*. *Proc. Zool. Soc. Lond.* part 1, 357–68.

ROTHSCHILD, A. & M. (1939). Some observations on the growth of *Peringia ulvae* in the laboratory. *Novit. zool.* **41**, 240–7.

ROTHSCHILD, M. (1936). Gigantism and variation in *Peringia ulvae* caused by infection with larval trematodes. *J. Mar. Biol. Ass. U.K.* **20**, 537–46.

ROTHSCHILD, M. (1941). Observations on the growth and trematode infections of *Peringia ulvae* in a pool in the Tannar Saltings, Plymouth. *Parasitology*, **33**, 406–15.

ULMER, M. J. (1951). *Postharmostomum helicis*; its life history, and a revision of the subfamily Brachylaeminae. *Trans. Amer. Micr. Soc.* **70**, 189–238, 319–47.

WESENBERG-LUND, C. (1931). Contributions to the development of trematode digenea. 1. Biology of *Leucochloridium paradoxum*. *K. danske vidensk. Selsk.* Afd. 9s, **4**, 90–142.

WESENBERG-LUND, C. (1934). Contributions to the development of trematode digenea. 2. Biology of fresh-water cercariae in Danish fresh-waters. *K. danske vidensk. Selsk.* Afd. 9s, **5**, 1–223.

EXPLANATION OF PLATE X

Fig. 1. *Agriolimax reticulatus*. Healthy renal tissue.
Fig. 2. *A. reticulatus*. Renal tissue with parasites.
Fig. 3. *Milax sowerbii*. Infested renal tissue with parasite.
Fig. 4. *A. reticulatus*. Renal tissue in the final stage of necrosis.

(*MS. received for publication* 6. v. 1957.—Ed.)

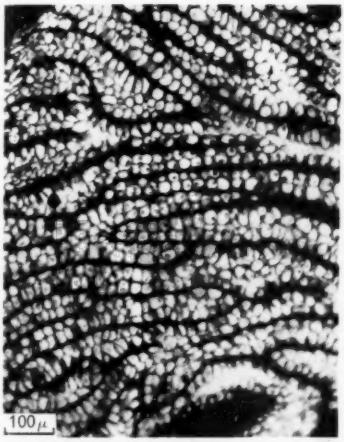


Fig. 1

Fig. 2

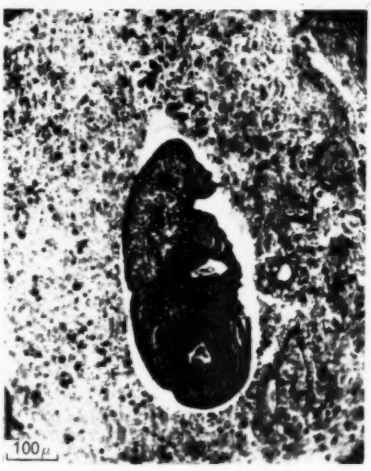


Fig. 3

Fig. 4

(*Facing p.* 268)

EXTERNAL FEATURES AND LIFE CYCLE OF *CUCUMARICOLA NOTABILIS* NOV.GEN. ET SP., A COPEPOD PARASITE OF THE HOLOTHURIAN, *CUCUMARIA*

By NELLIE F. PATERSON

Department of Zoology, University of Cape Town

(With 11 Figures in the Text)

Some time ago, while using preserved material of *Cucumaria frauenfeldi* Ludwig, obtained from Sea Point, Cape Town, for class purposes, several specimens were observed to contain one or more peculiar, amorphous cysts lying detached in the coelom and harmonizing so well with the viscera as to be almost indiscernible. As closer inspection of the contents of the cysts revealed the presence of an unusual and apparently unknown copepod parasite, an account of its occurrence and morphology may be of some interest.

Since the original findings in preserved hosts, examination of fresh material collected at intervals at Sea Point and Blaauwberg Strand on the Atlantic Coast and at Dalebrook, False Bay, has indicated a possible host specificity for the parasite, for, of the three common local species of *Cucumaria*, *C. sykion* Lampert, *C. stephensoni* John and *C. frauenfeldi* Ludwig, only specimens of the last-named species, have, so far, been found to harbour the parasites. All three species are fairly abundant at Dalebrook, but on the Atlantic side of the Cape Peninsula *C. frauenfeldi* is the only species found buried in sand-filled crevices of the rugged shore. Although perhaps no accurate estimate of the incidence of parasitism is to be gained from the number of hosts examined, it may be instructive to note that, of 106 collected at Sea Point and 202 at Blaauwberg Strand, only eleven and forty-nine respectively were parasitized. Random assemblage of 129 *Cucumaria* at Dalebrook produced twenty-nine *C. frauenfeldi*, of which only three were infected. Thus, although there are presumably fewer prospective hosts at Dalebrook, infection is subequal to that at Sea Point, while at Blaauwberg Strand, where the hosts are most plentiful, it is more than doubled.

Perusal of available literature indicates that, despite their sluggish habits and their apparent attraction for a variety of invertebrate commensals and parasites, the Holothuroidea are relatively free from infestation by copepods. Of some twenty genera known to be associated with echinoderm hosts, Wilson (1932) records only one harpacticoid, *Abacola*, and four cyclopoids, *Colaceutes*, *Diogenidium*, *Synapticola* and *Synaptiphilus*, in holothurians, and the only subsequent addition to this list seems to be *Scambicornus* (Heegaard, 1944), a cyclopoid on the tentacles of a Japanese holothurian. As far as the writer is aware, the present specimens have not hitherto been reported and they are totally unlike any copepod known to parasitize either echinoderm or other types of hosts. It therefore

seems advisable that a new genus be erected for their reception and, because they are noteworthy inhabitants of a species of *Cucumaria*, it is proposed to designate them *Cucumaricola notabilis*.

EXTERNAL MORPHOLOGY OF THE PARASITE

In common with the majority of known endoparasitic copepods of Echinodermata (Leigh-Sharpe, 1928*c*; Wilson, 1932; Stephensen, 1933, 1935), these specimens are found enclosed in galls or cysts, which vary in size from 0·5 to 30 mm. and are doubtless formed from host tissues. The smaller cysts, containing very young individuals, are spherical or oval structures invariably attached either to the tissues between the circumpharyngeal ossicles or to the inner wall of the cloaca near the respiratory trees. The larger cysts, of which only twenty were obtained, are amorphous and lie detached in the coelom; they contain mature parasites, the shape of the female being responsible for the irregular conformation of the cyst wall. Of the sixty-three parasitized *Cucumaria* collected, forty-five contained only one cyst, ten had two cysts, four had three, while in the remaining four hosts there were four cysts, the enclosed parasites of which varied so considerably in size as to suggest at least two or three successive invasions. Study of the animals removed from the cysts suggests that normally each cyst is destined to contain one pair of parasites, but occasionally it may contain two pairs or even only a single individual of either sex. The largest cysts also include numerous eggs in different stages of development and many fully formed nauplius larvae. The latter are still encapsuled by the egg membrane and provide important evidence relative to the early phases in the life history of the parasite. As immature and mature specimens were collected simultaneously, no decision could be reached as to the life-span of the parasite or the number of generations produced annually. Baer (1952) states that the majority of parasitic copepods have more than one breeding season and that some may reproduce continuously throughout the year. While neither of these events could be substantiated with certainty in the present species, the indications are that individuals are sexually mature in early spring and that oviposition is probably a continuous process, at least throughout spring and summer.

As frequently occurs in endoparasitic invertebrates, the present specimens have departed considerably from the morphological features of related free-living forms, the body being altered almost beyond recognition and the typical copepod appendages being either greatly modified or suppressed. Sexual dimorphism is very marked in the mature forms, the male being dwarfed by the female, which is six to eight times as large as her almost microscopic partner. In young individuals there is less disparity in size, but, even in the smallest specimens which are superficially almost identical in appearance, the two sexes may be distinguished by manifest dissimilarities in their appendages.

When alive, the mature animals are of a lustrous yellow or orange colour with the whitish oviducts of the female and the colourless vasa deferentia of the male clearly visible through the translucent body wall. In both sexes the elongate, cylindrical body (Figs. 1, 3) is divisible into a cephalothorax, a trunk of six segments and a pair of conspicuous, somewhat lanceolate, caudal rami. Judging by its appendages, the

cephalothorax represents the head plus the first thoracic segment, while the trunk is largely, if not entirely, the thorax. As the genital segment is terminal in both sexes, it would seem that the abdominal region is lacking, but its presence and also that of the caudal rami are variable quantities in other parasitic Copepoda, notably in the Lernaeopodoida (Wilson, 1915, 1932).

In many parasitic Copepoda the pygmy male is firmly adherent to its partner, but in the present species this close association of the sexes is not evident. It was observed, however, that young males with relatively short caudal rami are able to move with considerable rapidity over the exposed cyst wall by grasping the tissues with the claw-like antennae and supporting the body on the trunk appendages, the spinous processes of which provide a means of attachment and traction. Minute females, with short, stumpy appendages resembling those of the males, are also capable of locomotion, and in both sexes progression may be expedited by the execution of leech-like movements of the flexible trunk. The purpose of the uncinate maxillipeds of the male and their ostensible adaptation for prehension remains somewhat problematic, for in none of the individuals did they exhibit grasping movements when in contact with the cyst wall or the other partner. Fully mature specimens were invariably found lying apart within the cyst, the unwieldy female being apparently only capable of limited stretching movements of the body accompanied by alternate turgescence and relaxation of the appendages. The motility of the males, however, and the fact that, compared with known epigynic Copepoda, they are relatively large forms with a somewhat less accentuated sexual dimorphism, probably explains the separation of mature individuals. Furthermore, in respect of their powers of locomotion, the males recall those of the Pharodinae (Delamare Deboutteville, 1951), in which the two sexes, unlike those of related Chondracanthidae, are not permanently associated.

(*a*) *Male*

The males collected range in size from 0·51 to 4·5 or 5·0 mm. long, excluding the caudal rami (*c.r.*, Fig. 1), which are often erected dorsally and are always more than half the body length in mature specimens, but considerably shorter in adolescent individuals.

Superficially the male (Fig. 1) resembles an insect larva with a small head region demarcated from a cylindrical trunk, in which the six subequal segments are clearly delineated by annular grooves.

The *cephalothorax* (*ct.*, Figs. 1, 2A) is relatively small and slightly narrower than the trunk, being about 0·6 mm. long by 0·9 mm. across, as compared to a body width of 1·05 mm. It bears only three pairs of appendages, which are considered to be the antennules, the antennae and the maxillipeds. Close inspection of living and preserved specimens of both sexes revealed no convincing traces of mandibles or maxillae, but the atrophy of some appendages is apparently not unusual in parasitic Copepoda, although it is more compatible with the female than the male anatomy.

The *antennules* (Fig. 2B), which project from the antero-lateral margin of the cephalothorax, are short single-jointed structures, each with a small terminal spine adjacent to a truncated free extremity beset with several minute sensillae. The

better-developed *antennae* (Fig. 2C) are 3-jointed, the terminal article being acuminate and bearing a short fronto-medial spine. They are ventral in position and are directed posteriorly towards an area vaguely suggestive of a slightly chitinized oral region, although no definite aperture could be detected. In the morphological postoral region of the cephalothorax are the conspicuous 3-jointed *maxillipeds* (Fig. 2D) in which, as in many other Copepoda (Wilson, 1915), the terminal joints are arched and sharply flexed mesially over the swollen base. The latter is more

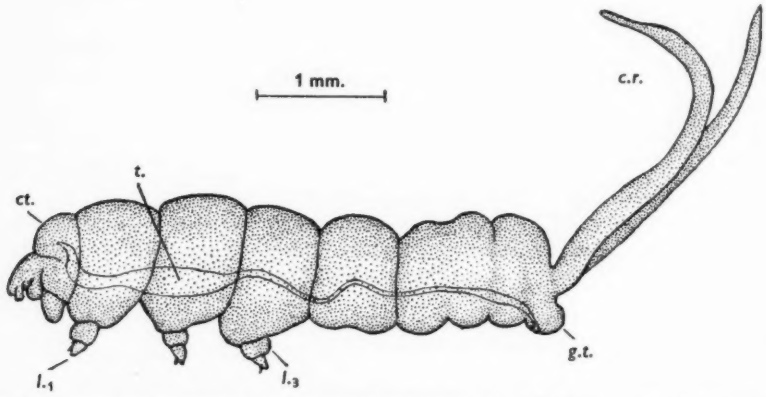


Fig. 1. Mature male parasite; side view, showing position of left reproductive organs.

Abbreviations used in the figures: a^1, antennule; a^2, antenna; *c.r.*, caudal rami; *ct.*, cephalothorax; *g.t.*, genital tubercle; l_1, l_2, l_3, three trunk appendages; *mxp.*, maxilliped; *od.*, oviduct; *o.s.*, ovisac; *s.r.*, seminal receptacle; *s.p.* spatulate process; *t.*, testis.

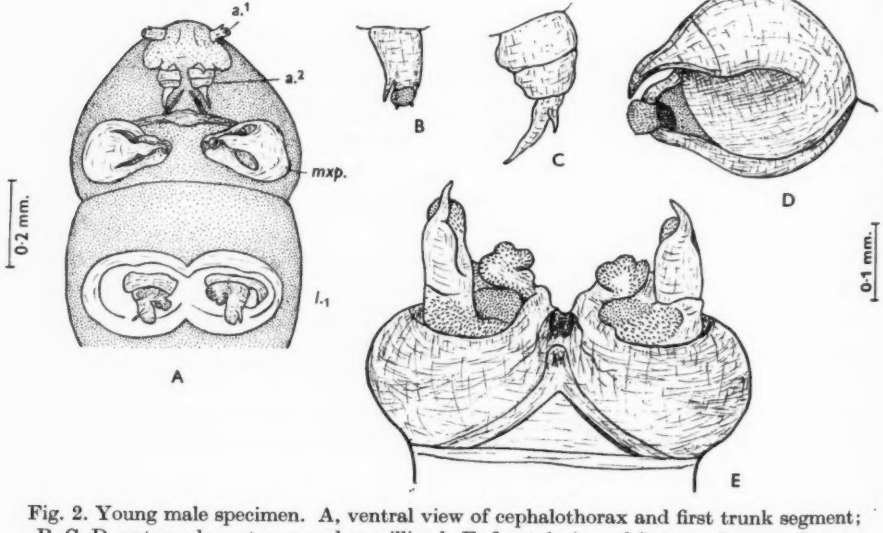


Fig. 2. Young male specimen. A, ventral view of cephalothorax and first trunk segment; B, C, D, antennule, antenna and maxilliped; E, frontal view of first trunk appendages.

strongly chitinized and furnished with a medial, roughened, socket-like depression, apparently for the reception of the terminal joint. Similar surfaces on the maxillipeds of other species, according to Wilson (1915), ensure a more secure attachment, while the spinous cushions on lernaeopodid maxillipeds described by Leigh-Sharpe (1926, 1928*a*) are probably identical in function. Thus, although there is no permanent union of the sexes, the similitude of the male maxillipeds with those of known epigynic forms suggests that they are occasionally used as prehensile organs.

Each of the first three *trunk* segments bears a pair of short, mid-ventral *appendages*, which are morphologically practically identical and unlike the usual copepod limbs. The swollen, ventrally projected, basal joints of each pair are in juxtaposition and almost confluent medially (Fig. 2E), perhaps foreshadowing the condition in the female, in which each pair of trunk appendages depends from a mid-ventral extension of its segment. Each large basal joint supports a short lateral shaft, which is indistinctly divided into two podomeres and is terminally bipartite with an anterior acuminate claw-like process partially covering a posterior truncated lobe. Basally and medially are three verrucose areas, one of which is finely setose and intercalated between the other two fields of rather coarse spinules. The verrucae are apparent even in the smallest males, and being distensible, they seem to assist in gaining purchase during locomotion.

Although no attempt was made to determine the internal anatomy by means of serial sections, it is evident from stained entire specimens that the gonads in both sexes are tubular enlargements at the anterior ends of the paired gonoducts. The testes (*t*, Fig. 1) are better defined than the ovaries and are located laterally in the first two or three trunk segments with their tapering extremities projecting a short distance into the cephalothorax. The slender vasa deferentia describe a somewhat sinuous course down the length of the body and become slightly wider as they converge posteriorly to open independently on the genital tubercle (*g.t.*, Fig. 1), which is conspicuous as a subspherical protuberance antero-ventral to the caudal rami.

(*b*) *Female*

While the youngest males and females are practically alike in size and appearance, during adolescence sexual dimorphism becomes evident, and ultimately, as in many other parasitic Copepoda, the adult female is peculiarly modified and considerably larger than the male. In its bizarre appearance it shows few distinctive copepod features, but a close examination of the parts nevertheless reveals its structural relationships with the male.

Owing to the marked curvature of the body, no precise measurements of the length of the females are possible, but approximate estimates show a range of from 0·8 to 20 mm. in juvenile and adolescent specimens and from 20 to 40 mm. in mature, fertilized forms. Additional to this are the caudal rami (*c.r.*, Figs. 3, 5), which are usually deflected and measure never more than one-sixth of the length of the trunk.

Proportionally the *cephalothorax* (Figs. 3, 4A, B) is smaller than that of the male, being 1·75 mm. long and 1·5 mm. wide, while the anterior width of the trunk is

about 2·3 mm. It resembles that of the male in having similar *antennules* and *antennae* (Fig. 4C, D), but differs in the atrophy of the *maxillipeds*. These are represented by a pair of bulbous protuberances (*mxp.*, Fig. 4B) situated in the morphological postoral region in a position directly comparable to that of the basal joints of the male maxillipeds. As in the male, the presumed oral region anterior to the maxillipeds is slightly chitinized, but there are no recognizable traces of mandibles or maxillae. Immediately postero-lateral to the antennae and presumably on the virtual cephalon, the female cephalothorax also shows another pair of subspherical, finely setose swellings (Fig. 4B). In certain species of parasitic Copepoda subjected to extreme simplification of the parts (Okada, 1927; Wilson, 1932) lobular expansions of the body have been identified as reduced appendages, but, in

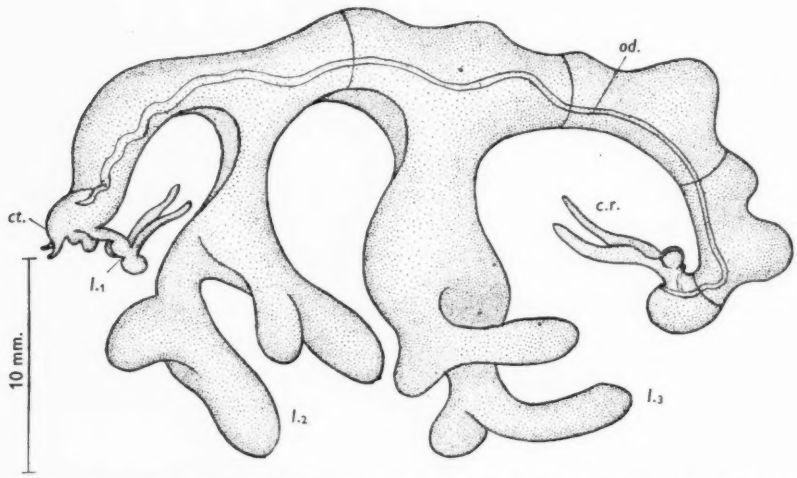


Fig. 3. Mature female; side view, with oviduct seen through translucent body wall.

view of observations on the postembryonic development, this is considered to be rather unlikely in the present specimens. Neither the first nor the second maxillae are apparent at any stage and, while the mandibles disappear in post-naupliar larvae, the maxillipeds only become evident in the initial juvenile parasite. Stained preparations of the latter show evidence of a simple alimentary tract, suggesting the possibility of their being capable of feeding while they penetrate the host tissues. This, however, is probably only a temporary measure, because there is no proof that the adults subsist on this type of diet. The suppression of the mandibles and maxillae would be consistent with their imprisoned condition and the obvious increase in the size of the cysts and their contained parasites suggests their dependence on the absorption of transudates from the host. The atrophy of the mouthparts is a feature of many Copepoda, but there is considerable variation in different families as to which of the cephalic appendages are retained. While many parasitic species may have the full complement of appendages, the poecilostome Cyclopoida (Lang, 1946–7, 1948) are characterized by the loss of the mandibles, a fact which is

further substantiated by Hockley's (1951) observations on the disappearance of the larval mandibles of *Mytilicola intestinalis* during metamorphosis. Such genera of the Lernaeopodoida as *Staurosoma* (Okada, 1927) and *Philichthys* (Wilson, 1932) are devoid of mouthparts, while one of the salient features of the adult Monstrilloida is the suppression, not only of the mouthparts, but also of the antennae.

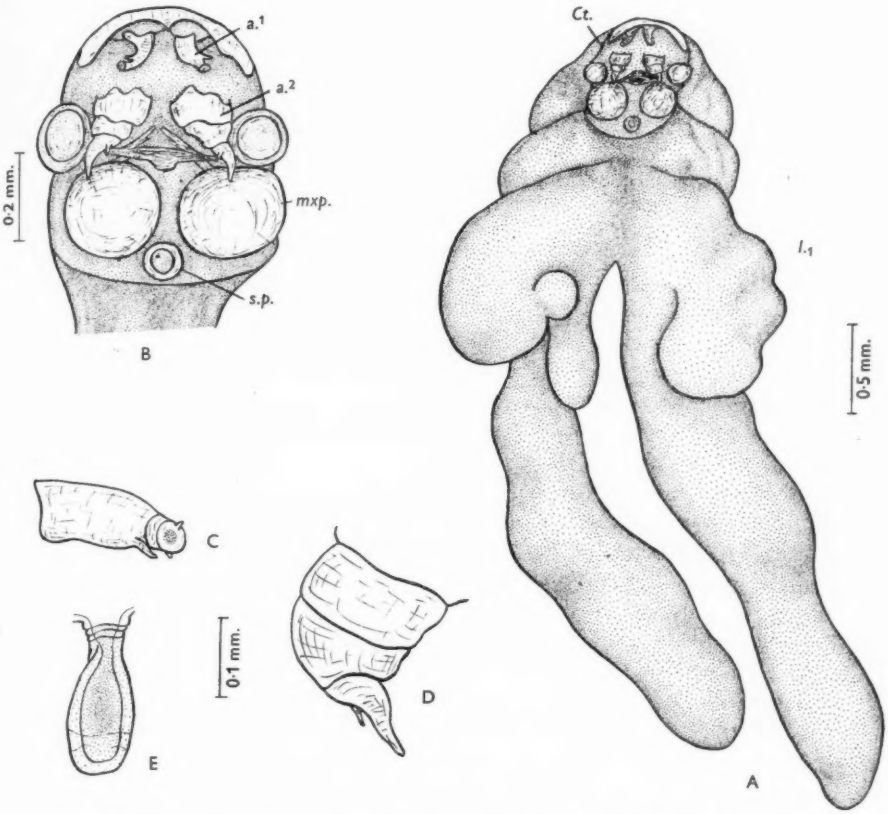


Fig. 4. Young female. A, ventral view of cephalothorax and first trunk appendages; B, ventral view of cephalothorax; C, D, E, antennule, antenna and spatulate process.

Posteriorly, the female cephalothorax bears a small median spatulate process (Fig. 4B, E) of uncertain homology. It is somewhat reminiscent of the bulla associated with the second maxillae of the Lernaeopodidae, in which, according to Leigh-Sharpe (1928*a*, 1930) and Wilson (1915, 1932), for the purpose of attachment to the host, the enlarged second maxillae have migrated behind the maxillipeds and their tips have fused with a bulla that develops from the larval frontal filament. In view of Friend's investigation of the metamorphosis of *Salmincola salmonea* (1941), this interpretation of the origin of the lernaeopodid bulla may have to be accepted with some reservation, for, in this particular species at least, the fertilized female

effects a secondary bullate attachment to the host independent of the larval filament and bulla. Quite apart from these divergent opinions regarding the development of the adult bulla, it is apparent from Wilson's review of the family (1915) and Leigh-Sharpe's descriptions of various species (1925, 1928, 1930) that there is a wide range in the size and shape of the second maxillae and bulla even within a single genus, with the result that, while some females are closely approximated to the gills of the host, others, due to the length of the maxillae, are projected at some distance from the surface of the host. Thus, whereas most species of *Clavella* have prominent maxillae fused terminally to the bulla, in *C. perfida* and *C. characis* the second maxillae have completely disappeared and the bulla persists as a relatively small stud protruding from the body. Admittedly there are no apparent structural similarities between adult Lernaeopodidae and the present specimens, but some significance seems to attach to the fact that the lernaeopodid bulla and the structure herein referred to as the spatulate process appear only in the female.

In the youngest, active females the three pairs of *trunk appendages* are short, bipartite structures superficially resembling those of the male, but lacking the medial rugosities and having a smaller anterior claw. They are subsequently converted into conspicuous, peculiarly shaped lobes (Fig. 3) which contribute largely to the grotesqueness of the female body. Each pair is suspended to a common mid-ventral outgrowth of its segment and each appendage takes the form of an unjointed, bilobed, somewhat boot-shaped organ, with the 'toe' of the 'boot' directed posteriorly. The first pair (Figs. 3, 4 A) is considerably smaller than the others and has become so closely applied to the cephalothorax that, at first sight, it appears to pertain to that region. In several species of parasitic Copepoda the trunk appendages are similarly modified into lobular extensions of the ventral body wall. This is particularly noticeable in *Staurosoma* (Okada, 1927) and in the chondracanthid subfamily Lernentominae (Leigh-Sharpe & Oakley, 1927), while it is perhaps of more interest that in the chondracanthid genera *Acanthochondria* and *Chondracanthopsis* Oakley (1930) and Wilson (1932) describe and figure two pairs of trunk appendages essentially similar to those of the present females.

The caudal rami of the female (*c.r.*, Figs. 3, 5) are not only markedly shorter than those of the male, but also differ in that each is basally dilated into a conspicuous tubercle immediately postero-lateral to the genital tubercle, the three nodules giving the appearance of tumid lips embracing the vulva. The genital tubercle is prominent in adolescent and young fertile females (*g.t.*, Fig. 5), but it seems to diminish with age. In the few egg-producing females collected it supports a pair of short, hyaline, filiform processes (Fig. 5) and, as these are associated only with fertile females in which there is an obvious sperm-filled seminal receptacle (*s.r.*, Fig. 5) antero-dorsal to the genital tubercle, it is surmised that they represent spent spermatophores. In other Copepoda spermatophores of varying size and shape are characteristically deposited close to the female vulva and in the Lernaeopodidae (Wilson, 1915; Gurney, 1934) they are firmly attached to a terminal genital process. The latter is probably reflected in the more nodular prominence of the present females and, apart from the aforementioned empty processes adherent to it, no other structures suggestive of spermatophores were seen on the surface of mature females.

In stained preparations the paired ovaries are not perceptibly differentiated from the oviducts, each of which (*od.*, Fig. 3) is a conspicuous convoluted tubule running laterally down the length of the body and terminating in a short transparent passage that receives a duct from the seminal receptacle before opening dorso-laterally close to the upper base of the caudal ramus of its side (Fig. 5).

Only one living female was observed to be ovigerous when freed from the cyst, but another female, which was maintained alive in the laboratory for 3 days, produced a pair of ovisacs on the second day, the eggs passing rapidly and singly down the transparent part of the oviduct into the ovisac. Each attached ovisac (*o.s.*, Fig. 5) is a delicate transparent capsule, measuring 1·25 mm. long by 0·75 mm. wide and enclosing numerous bright orange eggs. It was observed that shortly after

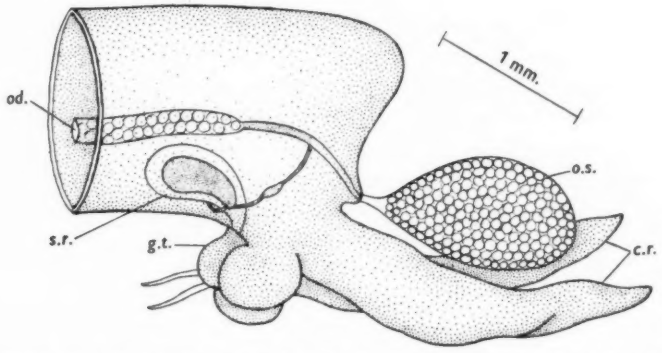


Fig. 5. Side view of posterior extremity of ovigerous female, showing left ovisac and pair of presumed spermatophores attached to genital tubercle.

completion the ovisacs become detached from the female and, as the mature cysts invariably contain a pair of slightly larger ovisacs, measuring 2·0 by 1·0 mm., in addition to numerous free eggs, it seems likely that the female not only produces a succession of ovisacs, but also that the eggs are subsequently dehisced into the cyst. The countless numbers of eggs in the cyst also suggest that egg-production is a continuous process throughout a relatively long breeding period and that the method of oviposition is merely a slight variation of that shown by other Copepoda. While the Notodelphyoida are characterized by a dorsal brood pouch, in the Arguloida and in some Calanoida the eggs are laid separately and undergo their development away from the parent. All the species of Copepoda previously recorded from echinoderm hosts belong to the Harpacticoida, the Cyclopoida or the Lernaeopodoida and bear characteristic ovisacs, but the occluded life of the present species has apparently resulted in the simple expedient of utilizing the cyst, after the liberation of a series of relatively small ovisacs, for the dual purpose of egg-receptacle and incubatory pouch.

(c) *Larval stages*

Because the mature cysts fortunately contain numerous fertilized eggs and advanced nauplius larvae, it has been possible to supplement the study of preserved material by observations on living specimens and thereby to gain some knowledge of postembryonic development and larval metamorphosis.

The bright orange eggs in the ovisacs measure 75μ in diameter, but, while they are undergoing cleavage in the cyst, the contents become pale yellow and the diameter of the enveloping membrane increases to 165–195μ. Although still retained in the hyaline egg membrane, the nauplii are substantially larger and measure 375–420μ long by 195–240μ broad. The body is filled with residual yolk, which apparently suffices as food material for the duration of larval life, as at no stage do the larvae seem capable of particulate feeding.

Each *nauplius* (Fig. 6) is more or less pyriform and bears three pairs of well-developed appendages, the segmentation of which is somewhat indistinct, but the disposition of transverse rows of spinules appears to coincide with the anterior limit of each joint. The apparently 5-jointed *antennules* (Fig. 7 A) are uniramous and terminate in a single long plumose seta, while dorsally there is a short basal seta. The *antennae* and *mandibles* (Fig. 7 B, C) are almost structurally alike, both being biramous and having four and two conspicuous plumose setae respectively on the exopodite and endopodite. They may, however, be distinguished by the fact that the antennary exopodite appears to be 6-jointed and bears a fifth inconspicuous distal seta, whereas the mandibular exopodite is composed of four articles. Ventrally between the antennules are two serrated ridges which may assist in rupturing the egg membrane and cyst wall. There is no trace of the characteristic median eye even in the colourless translucent body of living larvae, but its absence is not altogether exceptional for, according to Wilson (1915), in the Lernaeopodidae which have a similar development in the egg, the eye is usually rudimentary and, occasionally, as in *Achtheres*, it can be detected only in sectioned material.

Because there are obvious technical difficulties in culturing parasitic Copepoda, information regarding their ontogeny is rather meagre but, from the observations of Wilson (1911, 1915, 1922, 1932), Gurney (1931–33, 1934, 1945, 1947), Friend (1941), Heegaard (1947) and Lang (1948), it is apparent that there is a certain uniformity in the larval structural pattern, with which the nauplius here described compares very favourably. The main differences seem to relate to the suppression of the mandibles in certain species and to the number of appendicular joints and setae. Thus, while the nauplii of some species have only two pairs of limbs, the great majority further contrast with the present specimens in having at least two distal antennulary setae. The majority also have one or more pairs of caudal setae, termed balancers by Wilson (1915, 1922) and furcal setae by Gurney (1931–3, 1934). Although balancers have been shown by Wilson (1911, 1915) and Heegaard (1947) to be lacking in lernaeopodid nauplii, no such modification can be seen in the present species. Even in preserved material before removal of the egg membrane the caudal setae are visible as a pair of short hyaline processes diverging postero-laterally from the body. It is thus evident that these nauplii, although

confined within the egg membrane, bear a closer resemblance to those of free-living Copepoda than do the larvae of the Lernaeopodidae. This may be due to the fact that, even in the few Lernaeopodidae that have a free naupliar stage (Gurney, 1947; Heegaard, 1947), it is of very short duration and is succeeded almost immediately by the copepodid. Compared with these the present species is not only provided with an individual, less-restricted medium for the completion of its early

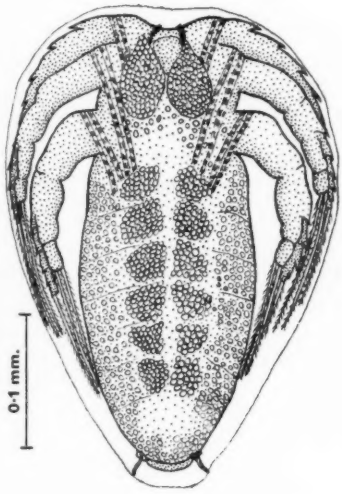


Fig. 6

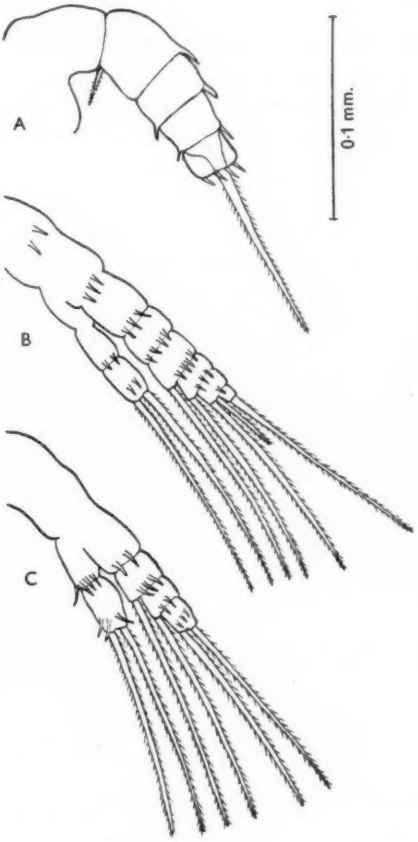


Fig. 7

Fig. 6. Ventral view of nauplius larva seen through the egg membrane.

Fig. 7. Appendages of nauplius larva. A, antennule; B, antenna; C, mandible.

development, but it is also apparent that the period spent as free-living larvae is longer.

Larvae in a more advanced state, but still enveloped by the egg membrane and also by what is probably a thin naupliar exuvia (Fig. 8), were also observed in the cysts. They measure 450 by 255μ and may correspond to the metanauplius of other forms but, as Heegaard (1947) indicates that this stage is probably present in few

parasitic Copepoda, it seems advisable to regard them merely as more fully developed nauplii. Essentially they resemble the younger larvae but, in addition to the naupliar cephalic appendages, they also possess the rudiments of the three pairs of the future copepodid trunk limbs.

While it is only possible to speculate as to the true course of events in nature, the following observations on larvae kept alive in the laboratory during the summer of this year have helped the elucidation of the main stages in the life cycle of the parasite. Despite an abundance of larval material in each mature cyst and because, no doubt, favourable culture conditions could not be provided, very few larvae were reared successfully to the juvenile parasite, the majority merely remaining alive for a week or 10 days in the early or late naupliar stages and showing no further progress. From this it seems that either some unknown factors govern the

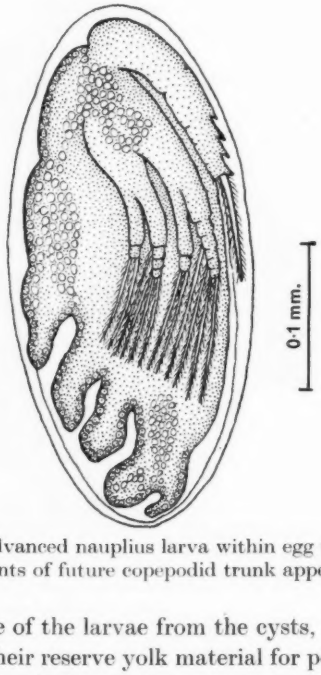


Fig. 8. Side view of advanced nauplius larva within egg membrane and showing rudiments of future copepodid trunk appendages.

precise time of emergence of the larvae from the cysts, or that they require some substance additional to their reserve yolk material for post-naupliar development.

In attempting to find a suitable medium, unruptured cysts and isolated larvae were immersed in filtered sea water or in sea water to which one of the following had been added: 0·25 % dextrose, 0·5 % dextrose, 0·1 % peptone or extract of the coelomic fluid of the host. In all cases the medium was renewed daily and was constantly aerated as a means of agitating the water. The containers were either placed in the cold rooms of the laboratory at temperatures of 13°–16° C. or were kept under observation at ordinary room temperatures of 20–25° C. and, although no conclusive results were obtained, it was noted that the larvae remained alive longer in sea water than in dilutions of dextrose or peptone. In view of Heegaard's experiences in culturing other parasitic Copepoda (1947), the conclusion that tem-

perature seemed to have no appreciable effect on the survival of the larvae was rather unexpected, but it may perhaps be associated with the fact that parasitized hosts occur on both sides of the Cape Peninsula, where the differences in water temperature are considerable. The only larvae that completed their development were a few of many introduced into diluted coelomic fluid of *Cucumaria* and, while this circumstance may have been purely fortuitous, no difference could be detected between these particular larvae and others from the same cyst that failed to develop further or only reached the first copepodid stage in other media.

The relatively thin cyst wall is soft and translucent and kept fairly taut by the cumbersome body of the female, slight movements of which seem to effect a slow circulation of the contained eggs and developing larvae. The advanced stages evidently move to the periphery and become pressed against the cyst wall, whence they were observed to force their way vertically through the surface. After escaping from the cyst they remain quiescent for a short period and then, preparatory to darting through the water, they execute a few quivering movements of their appendages. With the antennules held porrect, they ascend spasmodically in a counter-clockwise spiral until the surface is reached, subsequently falling inertly to the bottom with the caudal setae projecting laterally from the body and serving to break the fall. Presumably in escaping from the cyst the nauplii also rupture the egg membrane and then undergo rapid post-naupliar transformation for, not only did the majority become active immediately on being immersed in the culture medium, but copepodid larvae were observed within 16–24 hr. and a few juvenile parasites within 48 hr. The number of naupliar ecdyses was not established, the majority of the larvae moulting only once and failing to develop further. As, however, in one instance a copepodid stage was seen with four telescoped naupliar exuviae still attached, it seems likely that several brief naupliar stages may precede the copepodid.

Transformation of the nauplius into the copepodid is accomplished not only by the elongation of the body and the exposure of the trunk appendages, but also by the disappearance of the naupliar mandibles and the modification of the antennules and antennae. There seem to be only two copepodid stages, both essentially alike in appearance and differing mainly in the inertia that follows on ecdysis into the second stage.

The first *copepodid larva* (Fig. 9), which measures 457 to 495μ long by 165μ across the cephalothorax, is an active form capable of swimming vigorously in counter-clockwise spirals to the surface and regaining the bottom by sinking head-first through the water. Its body consists of a broad cephalothorax, roughly one-third of the total length, and a tapering trunk of five clearly demarcated segments terminating in a pair of caudal rami, each of which bears one long and two short setae.

In addition to the short paired antennules and antennae, it possesses three pairs of forwardly directed, biramous trunk appendages, the first pair attached to the last somite of the cephalothorax and the other two to the first two free trunk segments. The antennules and antennae (Fig. 9C, D) are markedly different from those of the nauplius larva, but the trunk appendages (Fig. 9E, F) are typical

copepod natatory limbs. Apart from the fact that the rami of the first two pairs are 2-jointed, while those of the last pair (Fig. 9F) are unsegmented, the limbs are similarly constructed. The exopodites and endopodites are broad and foliaceous and furnished with relatively long setae, a few of which are spatulate and have hyaline serrated margins. The maxillipeds are not, as yet, apparent and, probably because there is no visible median eye, no definite response to light was observed.

After an active existence of 24 hr. or less the larva settles on the bottom and moults into a slightly larger (540 by 190 μ) *second copepodid stage*. This is a quiescent

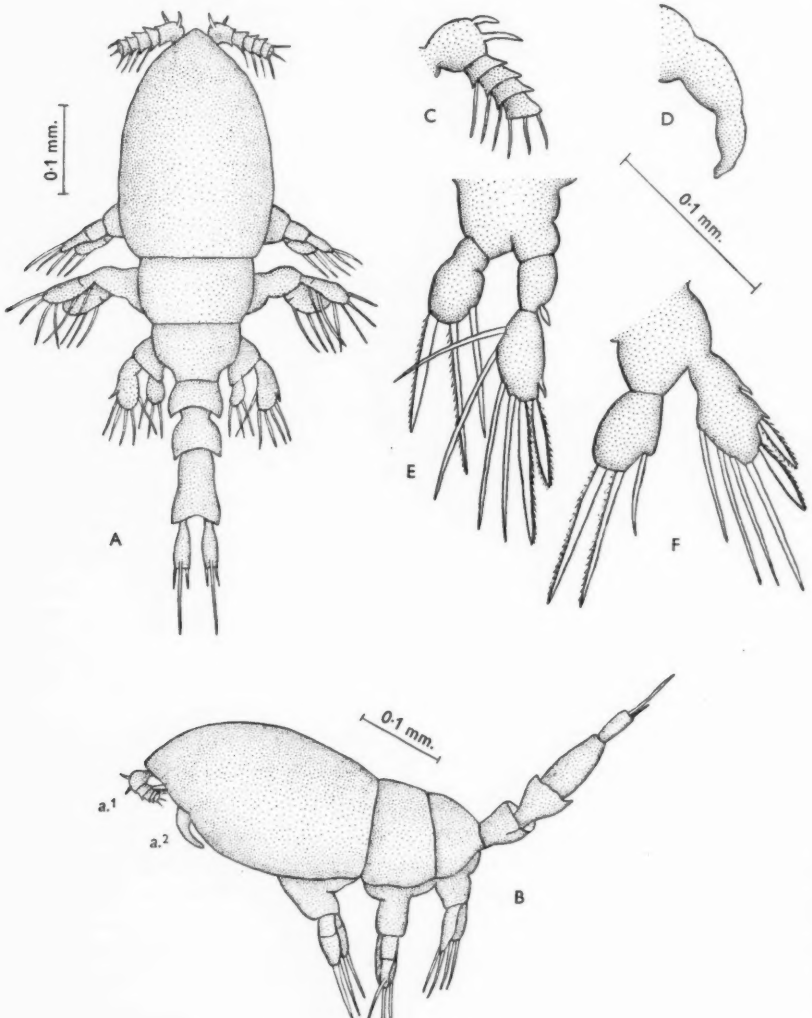


Fig. 9. First copepodid larva. A, dorsal view; B, side view; C, antennule; D, antenna; E, second trunk appendage; F, third trunk appendage.

phase in which the trunk appendages are directed posteriorly and during which transformation into the juvenile parasitic form takes place. It may be assumed that this represents the normal procedure for, in seeking the bottom, the copepodid and subsequently the young parasite would be brought into closer proximity with their more or less sedentary host.

The body and appendages of the *juvenile parasite* (Fig. 10) develop in direct relation to the parts of the copepodid and, on emerging anteriorly from the ruptured copepodid skin, the minute vermiform creature begins to crawl on the bottom, performing leech-like movements similar to those of small forms isolated from the host. The main morphological changes attendant on metamorphosis into the parasitic stage are the exposure of the bulbous maxillipeds and the modification of the three pairs of copepodid swimming appendages into short stumpy pereiopods. Although it was impossible to determine the sex of the minute parasites cultured

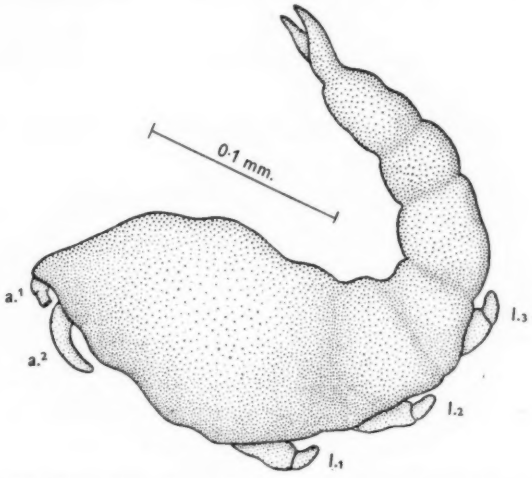


Fig. 10. Side view of juvenile parasite cultured experimentally.

experimentally, apart from their smaller size and less perfect segmentation, they differ so little from the juveniles found in the host (Fig. 11) that it may be supposed that at this stage they seek out and actively enter the host. As sexual differences are recognizable even in the copepodid stage of some species, the indeterminate nature of the copepodid larvae and the initial parasitic form suggests that in *Cucumaricola* sex-determination may be delayed until the small individuals make contact with the host. This would account for the almost complete lack of sexual dimorphism in the youngest parasites and for the motility of the smallest females. Once they are established in a cyst, the trunk appendages of the female gradually enlarge and change in shape and, with the elongation of the body in both sexes, the cephalothorax becomes proportionally smaller and better demarcated from the segment bearing the first pair of trunk appendages.

It seems rather significant that the smallest individuals of both sexes are essentially paedomorphic and that in the male (Figs. 1, 11), beyond an increase in size,

maturation evokes little structural change. This is not only consistent with Baer's statement (1952) that some male parasitic Copepoda are neotenic, but also corroborates Gurney's conclusions (1934, 1945) that Copepoda such as the Lernaeopodidae attain sexual maturity at a stage corresponding to the first or second copepodid larva of other species. Although agreeing with the Lernaeopodidae in this respect, the stages in the present species bear little true resemblance to those of known lernaeopodids. One major difference, the lack of the frontal filament, is sufficient in itself to warrant their removal from the Lernaeopodoida and relate them to the Cyclopiformes, a group suggested by Oakley (1930) for the inclusion of some free-living forms and the parasitic Ergasilidae, Monstrillidae and Chondracanthidae. Furthermore, with the possible exception of *Lernaeopoda galei* (Gurney, 1947), the lernaeopodid nauplius has no mandibles and their copepodids characteristically possess only two pairs of fully developed natatory appendages.

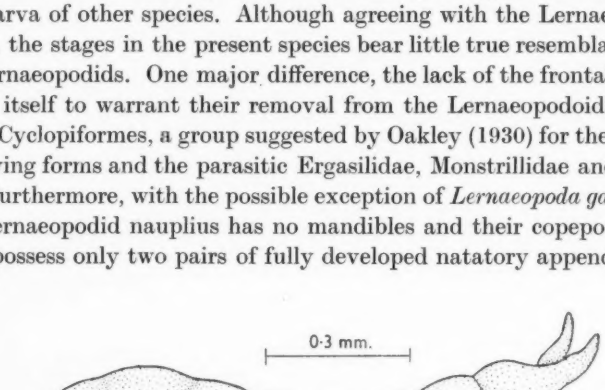


Fig. 11. Side view of small male removed from host.

On the basis of the foregoing observations it is reasonable to conclude that the life history is a fairly simple one involving the production of prodigious numbers of eggs and larvae inside the host and that, when the advanced nauplius stage is reached, the parasites emerge for a brief free-living existence as copepodid larvae before they effect entry into another host. The problem of how they enter and leave the host remains unsolved, but the discovery of two minute individuals apparently in transit through the wall of the pharynx and the attachment of the small cysts to the pharynx and cloaca suggest that the orifices of the host offer easy portals of entry. The delicate respiratory trees and the expiratory currents of water may provide a route of escape once the larvae have emerged from the cysts into the coelom of the host, but, however their dispersal is accomplished, it evidently entails a high rate of mortality, because the fecundity of the females is vastly disproportionate to the incidence of parasitism and the number of hosts available.

SUMMARY OF DIAGNOSTIC FEATURES OF THE NEW GENUS, *CUCUMARICOLA*

Occurrence. The name *Cucumaricola notabilis* is proposed for a copepod parasitic in *Cucumaria frauenfeldi* Ludwig collected in the vicinity of Cape Town. Of sixty-three parasitized hosts assembled, eleven were from Sea Point and forty-nine from

Blaauwberg Strand on the Atlantic coast of the Cape Peninsula, while the remaining three hosts were collected at Dalebrook, False Bay. The description of the type is based on material from Blaauwberg Strand where the hosts were most plentiful and the incidence of parasitism highest. From this locality living juvenile, adolescent and mature parasites, as well as nauplius larvae, were obtained during the spring and summer months of 1956–7.

Sexually dimorphic individuals occur usually in pairs in cysts in the coelom of the host. The body in both sexes is cylindrical translucent yellow when alive, and composed of a small cephalothorax, a trunk of six segments and a pair of laminate caudal rami.

Holotype: mature male. The body is 4·0 mm. long, with caudal rami conspicuous and more than half body length. Cephalothorax with paired short uniramous antennules, prehensile antennae and uncinate maxillipeds. No mandibles or maxillae. Trunk segmentation quite well defined. Three pairs of short trunk appendages, modified as pereiopods.

Holotype: mature female. The body is arched and approximately 35 mm. long. Cephalothorax relatively small, bearing antennules and antennae similar to those of male, but maxillipeds represented by bulbous protuberances. Each of the first three trunk segments with a pair of clumsy boot-shaped appendages, the first approximated to the cephalothorax. Caudal rami shorter than those of male and about one-sixth of body length. Type not ovigerous, but two other females from same locality produced small, paired, oval ovisacs containing numerous bright orange eggs.

Life cycle. The ovisacs are serially produced and become detached from the female shortly after completion. The eggs are then dehisced into the cyst, in which development proceeds within the egg membrane to the late nauplius. In experimental cultures the nauplii were observed to escape from the cyst and metamorphose into two copepodid stages in 16–48 hr. The first copepodid is an active swimming form, while from the second, quiescent copepodid, juvenile parasites emerge about 48 hr. after the nauplii become free of the cyst. Both sexes of young parasites are active, creeping forms, almost identical in size and appearance. Presumably they enter the host almost immediately, because very small cysts attached to the pharynx and cloaca of the host contain similar individuals. On maturation of the parasites, the cysts become detached and lie in the coelom, entangled in the host's viscera.

Genotype. Cucumaricola notabilis, new species, deposited in the Department of Zoology, University of Cape Town.

SYSTEMATIC POSITION

Existing classifications of Copepoda based on adult features are admitted by most authorities to be imperfect because, while there may be general agreement concerning the phylogeny of most free-living forms, some of the parasitic species are morphologically so aberrant that their systematic position is obscure. Furthermore, in many of them the males are unknown and the classification of the highly specialized females is consequently somewhat tentative.

Leigh-Sharpe has made the interesting suggestion (1928*c*, p. 424) that 'there is a

definite correspondence between the degree of degeneracy exhibited by the parasite and the distribution of its host in time', and on this basis he ranks the Echinodermata second to the Annelida as the hosts harbouring the most degenerate copepod parasites. His analysis, however, shows little discrimination between ectoparasites and endoparasites, and hardly accounts for the morphological variations of the parasites and the diversity of hosts associated with some of the orders. In the least degenerate category he places the copepods of more recent fishes and ascidians but, in apparent contradiction to this, he cites as one of the most degenerate families the Lernaeopodidae, which are fish parasites. In this connexion it may be noted that, in addition to the many species that infest elasmobranch and teleostean fishes, the order Lernaeopodoida (Wilson, 1932) also contains others associated with Anthozoa, Annelida, Crustacea, Echinoidea, Ophiuroidea and Ascidiacea. Even in the essentially free-living Cyclopoida, Monod & Dollfus (1932) and Wilson (1932) record numbers of ectoparasites and endoparasites in hosts belonging, not only to the assorted groups frequented by Lernaeopodoida, but also to the Mollusca and all classes of Echinodermata. While the majority are host-specific and the species in each genus are usually attracted to related hosts, a few seem to have adapted themselves to more than one host and occasionally affiliated species may select vastly different hosts. Thus, for instance, in the genus *Philichthys* one species occurs in the mucous canals of the swordfish *Xiphias gladius*, while another is parasitic in the ophiuroid *Amphipholis squamata*. As previously indicated, some twenty genera of Copepoda are associated with echinoderm hosts and, although the majority are endoparasitic, only a few, such as *Arthrochordeumium* and *Ophioika* (Stephensen, 1933; Pyefinch, 1940), have become profoundly modified, the latter so much so that it is hardly recognizable as a copepod. From this it may be assumed that, while morphological simplification in copepods may sometimes be correlated with the geological age and systematic position of the host, it seems to provide no conclusive evidence concerning the affinities of the parasites themselves.

Morphologically *Cucumaricola* bears no close resemblance to any known copepod parasite, but the presence of sexual dimorphism, the prehensility of the antennae and the resemblance of the unusual trunk appendages of the female to those of *Acanthochondria* (Oakley, 1930; Wilson, 1932) suggest a possible relationship with the subfamily Chondracanthinae of the family Chondracanthidae. In other respects *Cucumaricola* is totally unlike the Chondracanthinae, but the major differences may relate to dissimilar habits. All the Chondracanthinae are parasitic on the gills of fishes, and to obtain their presumed mixed diet of blood and tissues (Oakley, 1930) they are equipped with definite mouth parts. Contrasted with this, the encysted endoparasitic existence of *Cucumaricola* seems to have resulted in the suppression of a feeding apparatus, the absence of which creates difficulties in attempting to correlate the species. Another difference that may be recalled is that the males of Chondracanthinae are more or less permanently epigynic, whereas in the Pharodinae (Delamare Deboutteville, 1951) and also in *Cucumaricola* they are motile and not adherent to the females. On the basis of this and certain structural characteristics, Delamare Deboutteville (1951) considers that the Pharodinae are the most primitive of the Chondracanthidae and that they bridge the gap between

this family and the Splanchnotrophidae. The latter, which comprises several species of the genus *Splanchnotrophus* found in nudibranch molluscs, seems to be a somewhat puzzling family; but it is related by Monod & Dollfus (1932), Gurney (1933), Delamare Deboutteville (1951) and Delamare Deboutteville & Nunes-Ruivo (1955) to the Chondracanthidae. It is also of interest to note that *Staurosoma*, an anthozoan parasite tentatively linked with this family by Okada (1927), is regarded by Delamare Deboutteville & Nunes-Ruivo (1955) as being akin to the Chondracanthidae, and it is possible that other aberrant genera included by Wilson (1932) in the Lernaeopodoida may have similar claims.

The position of the Chondracanthidae thus seems to be relevant to the determination of the affinities of *Cucumaricola* and other genera of uncertain lineage. In this connexion there is increasing support for the removal of this family from Wilson's Lernaeopodoida (1932) and its association with the Ergasilidae in Oakley's Cyclopiformes (1930). In supporting Oakley's contentions, Gurney (1931–3) suggests that all parasitic Copepoda may have a possible origin in the Ergasilidae, to which the Chondracanthidae appear to be more closely related than Wilson (1932) implies. Gurney further indicates that the position of the Lernaeopodidae is somewhat isolated and that the general resemblance of their dwarf males to those of Chondracanthidae may result from arrested development and consequent paedomorphism in both families. Paedogenesis, however, occurs in many parasitic and semiparasitic copepods and, as has been indicated, is applicable to both sexes of *Cucumaricola*. In this case the further elaboration of the female body hardly obscures the features common to both sexes and, while this may also be true of other species, the adults of the majority are so variable that the classification of some of the groups is founded on rather superficial characteristics.

The desirability of helping the clarification of the systematics of the Copepoda by supplementing comparative morphological studies of the adults with information on the ontogeny of the species has been expressed on several occasions by Gurney (1931–3, 1934, 1945), but it is obvious that this cannot be realized in many endoparasites. It is, nevertheless, significant that Wilson (1911*a*) and Baer (1952), in analysing the life cycles of parasitic species, relegate the Chondracanthidae and Lernaeopodidae to different categories on the basis of the particular stage or stages of free-living existence and their duration. In both families the life history is shortened by the suppression of some of the stages but, while all the larval phases of the Chondracanthidae are usually non-parasitic, the copepodid is typically the only free-living stage in the Lernaeopodidae. Post-embryonic development in the latter family also seems to be subject to slight variations for, while Wilson (1911*a*) states that some may hatch as metanauplii, Gurney (1947) reveals that certain species have a free, but not necessarily a swimming, nauplius that transforms almost immediately into a copepodid. It would thus seem that occasionally there is little effectual difference in the ontogeny of the two families, a fact which to some extent is analogous to the confusion relating to the phylogeny of the families expressed on the basis of adult features. These difficulties are, however, largely resolved by Heegaard's illuminating studies of the life histories of several parasitic species (1947), from which it emerges that parasitic Copepoda may be divided into two

groups, the *Pectinata* and the *Fistulata*. Among other factors, the latter are distinguished by the lack of a frontal filament and by the 2-jointed endopodite of the naupliar mandible. The Chondracanthidae, which belong to this group, are thus related to the Ergasilidae and are distinctly separated from the Caligidae, Lernaeidae and Lernaeopodidae contained in the *Pectinata*.

Heegaard's account (1947) of *Acanthochondria cornuta* seems to be the first comprehensive description of the development of a chondracanthid and is particularly pertinent to the present observations. It has been indicated that the distinctive anatomical and developmental characteristics of *Cucumaricola* serve to isolate it from other forms and, as both sexes lack the typical copepod facies, attempts to relate it to known parasitic species must be of a tentative nature. It may be conceded, however, that it has little morphological or ontogenetic claim to inclusion in Wilson's Lernaeopodoida (1932), although this is admittedly a somewhat heterogeneous group containing a number of genera of questionable affinities. As far as is known, it includes only two echinoderm parasites, *Philichthys amphiurae* Hérouard in the ophiuroid *Amphipholis squamata* and *Pionodesmotes phormosomae* Koehler in the echinoid *Hygrosoma petersi*, the former probably having a more natural setting in the Cyclopiformes and neither showing any resemblance to *Cucumaricola*. Moreover, the life cycle of *Cucumaricola* is too unlike that of Lernaeopodoida to admit of any real homology. On the other hand, while the slight resemblance between the adult females of *Cucumaricola* and *Acanthochondria* can hardly be regarded as adequate proof of interrelationship, if the emphasis be placed on development it seems clear that *Cucumaricola* has a positive identity with Heegaard's *Fistulata* (1947) and thus with Oakley's Cyclopiformes (1930). Not only is the mandibular endopodite of the nauplius of *Cucumaricola* 2-jointed, but the presence of a fifth short seta on the naupliar antennary exopodite and the general similarity of the copepodid larva to that of *Acanthochondria* seem to support the opinion regarding the approximation of the adult females. It is also possible that in the retention of the third pair of trunk appendages the copepodid of *Cucumaricola* shows a greater similarity to that of the Ergasilidae (Gurney, 1933) than does the larva of *Acanthochondria*. In its lack of mouthparts, however, the genus is sharply differentiated from the majority of Cyclopiformes, but an approach to the modifications in the Ergasilidae described by Wilson (1911*b*) and Gurney (1933) may be evinced in the arrangement and function of the antennules and antennae and in the suppression of the maxillipeds in the adult female. If this supposition is at all feasible, then it should lend support to the derivation of parasitic forms such as the Chondracanthidae from the more or less semiparasitic Ergasilidae. At the same time, the absence of mouthparts may also reflect the condition in *Philichthys* and *Staurosoma*, relevant to which it is of importance to note that, in reviewing the features of a number of perplexing genera, Delamare Deboutteville & Nunes-Ruivo (1955) associate the Philichthyidae, Staurosomidae, Splanchnotrophidae and their new family, Echiurophilidae, with the Chondracanthidae. As the affinities of *Cucumaricola* also seem to point towards this family, it is reasonable to conclude that it may appropriately be appended as a new genus, if not a new family, to their key for chondracanthid-like copepods parasitic in invertebrate hosts.

SUMMARY

1. *Cucumaricola notabilis*, a new genus and species of Copepoda, occurs in cysts in the coelom of *Cucumaria frauenfeldi* Ludwig collected at Sea Point, Blaauwberg Strand and Dalebrook in the Cape Peninsula.

2. An account is given of the features of the sexually dimorphic males and females.

3. Fertilized eggs and nauplius larvae are also contained in the cysts and, from observations on living material, it has been established that, after escaping from the cysts, the larvae metamorphose within a short period into the copepodid stage. There are two copepodid stages, the first an active swimming form and the second a quiescent phase from which the juvenile parasite develops.

4. Although of uncertain systematic position, this parasite seems to be nearly related to the Chondracanthidae. This conclusion is based on the similarity of the trunk appendages of the adult female to those of *Acanthochondria* as described by Oakley (1930), and also on the general agreement of the ontogeny with that of *Acanthochondria cornuta* investigated by Heegaard in 1947. It is further suggested that it may be assigned to the key devised by Delamare Deboutteville & Nunes-Ruivo (1955) for certain copepod parasites having some affinity with the Chondracanthidae.

REFERENCES

Baer, J. G. (1952). *Ecology of Animal Parasites.* Urbana: Illinois University Press.

Delamare Deboutteville, Cl. (1951). Présence de la sous-famille des Pharodinae Illg (Copépodes, Chondracanthidae) en Méditerranée et remarques sur la classification et la biologie des Chondracanthidae. *Arch. Zool. exp. gén.* **87**, 139–55.

Delamare Deboutteville, Cl. & Nunes-Ruivo, L. (1955). *Echiurophilus fizei* n.g. n.sp., copépode parasite d'un échiuride d'Indochine. *Vie et Milieu*, **6**, no. 1, 101–12.

Friend, G. F. (1941). The life-history and ecology of the salmon gill-maggot, *Salmincola salmonea* (L.) (Copepod Crustacean). *Trans. Roy. Soc. Edinb.* **60**, pt. II, 503–41.

Gurney, R. (1931–3). *British Fresh-Water Copepoda*, 3 vols. Ray Soc. London.

Gurney, R. (1934). The development of certain parasitic Copepoda of the families Caligidae and Clavellidae. *Proc. zool. Soc. Lond.* pp. 177–217.

Gurney, R. (1945). Some notes on the development and classification of parasitic Copepoda. *Ann. Mag. nat. Hist.* (11), **12**, 121–7.

Gurney, R. (1947). Some notes on parasitic Copepoda. Developmental stages in the family Lernaeopodidae. *J. Mar. Biol. Ass. U.K.* **27**, 133–7.

Heegaard, P. (1944). A new copepod (*Scambicornus hamatus*) parasitic on a Japanese holothurian. *Vidensk. Medd. dansk. naturh. Foren. Kbh.* **107**, 359–66.

Heegaard, P. (1947). Contribution to the phylogeny of the arthropods: Copepoda. *Spolia zool. Mus. Hauniensis*, **8**, 1–227.

Hockley, A. R. (1951). On the biology of *Mytilicola intestinalis* (Steuer). *J. Mar. Biol. Ass. U.K.*, **30**, 223–32.

Lang, K. (1946–7). A contribution to the question of the mouth-parts of copepods. *Ark. Zool. Stockh.* **38** A, no. 5, 24 pp.

Lang, K. (1948). Copepoda 'Notodelphyoida' from the Swedish West Coast, with an outline on the systematics of the copepods. *Ark. Zool. Stockh.* **40** A, no. 14, 36 pp.

Leigh-Sharpe, W. H. (1925). A revision of the British species of *Clavella* (Crustacea, Copepoda), with a diagnosis of new species, *C. devastatrix* and *C. invicta*. *Parasitology*, **17**, 194–200.

Leigh-Sharpe, W. H. (1926). *Ommatokoita superba* n.g. et sp., a parasitic copepod of *Somniosus microcephalus*. *Parasitology*, **18**, 224–9.

LEIGH-SHARPE, W. H. & OAKLEY, C. L. (1927). Lernentominae, a new subfamily of Chondracanthidae (Crustacea: Copepoda), with a description of *Oralien triglae* Blainville. *Parasitology*, **19**, 455–67.

LEIGH-SHARPE, W. H. (1928*a*). *Brachiella obesa*, a parasitic copepod of *Trigla cuculus*, with a description of the male. *Parasitology*, **20**, 25–31.

LEIGH-SHARPE, W. H. (1928*b*). The genera *Charopinus* and *Lernaeopodina* (Copepoda) as represented by the collection in the British Museum. *Parasitology*, **20**, 273–9.

LEIGH-SHARPE, W. H. (1928*c*). Degeneracy in parasitic Copepoda in relation to the distribution of their hosts in time. *Parasitology*, **20**, 421–6.

LEIGH-SHARPE, W. H. (1930). Further remarks on the genus *Lernaeopoda*, including a description of a new species, *L. bivia*. *Parasitology*, **22**, 137–42.

MONOD, TH. & DOLLFUS, R. PH. (1932). Les copépodes parasites de mollusques. *Ann. Parasit. hum. comp.* **10**, 129–204.

OAKLEY, C. L. (1930). The Chondracanthidae (Crustacea: Copepoda), with a description of five new genera and one new species. *Parasitology*, **22**, 182–201.

OKADA, Y. K. (1927). *Staurosoma*, copépode parasite d'une actinie: description de *Staurosoma caulleryi* sp.n. *Annot. zool. jap.* **11**, 173–81.

PYEFINCH, K. A. (1940). The anatomy of *Ophioica asymmetrica* sp.n., a copepod endoparasitic in an ophiuroid. *J. Linn. Soc. Zool. Lond.* **41**, 1–19.

STEPHENSEN, K. (1933). Some new copepods, parasites of ophiurids and echinids. *Vidensk. Medd. dansk. naturh. Foren. Kbh.* **93**, 197–213.

STEPHENSEN, K. (1935). Some endoparasitic copepods found in echinids. *Vidensk. Medd. dansk. naturh. Foren. Kbh.* **98**, 221–8.

WILSON, C. B. (1911*a*). North American parasitic copepods. Part 9. The Lernaeopodidae. Development of *Achtheres ambloplitis* Kellicott. *Proc. U.S. Nat. Mus.* **39**, 189–226.

WILSON, C. B. (1911*b*). North American parasitic copepods belonging to the family Ergasilidae. *Proc. U.S. Nat. Mus.* **39**, 263–400.

WILSON, C. B. (1915). North American parasitic copepods belonging to the Lernaeopodidae, with a revision of the entire family. *Proc. U.S. Nat. Mus.* **47**, 565–729.

WILSON, C. B. (1922). North American parasitic copepods belonging to the family Dichelesthiidae. *Proc. U.S. Nat. Mus.* **60**, Art. 5, 98 pp.

WILSON, C. B. (1932). The copepods of the Woods Hole region, Massachusetts. *Bull. U.S. Nat. Mus.* **158**, 1–635.

(*MS. received for publication* 13. v. 1957.—Ed.)

GLOBIDIUM GILRUTHI (CHATTON, 1910) INFECTION IN THE DIGESTIVE TRACT OF SHEEP AND GOATS IN EGYPT

By K. N. SOLIMAN, Ph.D., D.V.M.&S.*

Veterinary Research Institute, Ministry of Agriculture, El Dokki, Giza, Egypt

The main object of this short paper is to record the occurrence of *Globidium gilruthi* (Chatton, 1910) in sheep and goats in Egypt.

On the 24 January 1955 a yearling ewe, previously dosed with eggs of *Taenia multiceps* from an experimentally infected pup, was killed in order to follow the course of the infection. On post-mortem examination, apart from the other parasitological findings met with as a direct result of the experiment, the abomasum revealed the existence of a number of round, white cysts embedded in the mucous membrane, mainly in the region of the fundus. Some of these cysts were empty and looked like small pinhead ulcers with their circumferences whitish and raised. Thorough examination of the small intestine showed that the duodenum, jejunum and ileum were also infested. The posterior third of the small intestine was less severely infested than the anterior two-thirds. The cysts had a diameter of $\frac{1}{2}$–1 mm. and their centres were slightly raised above the level of the surrounding healthy mucous surface.

Examination of the faeces showed that there was no coccidiosis and no coccidia were found in scrapings from the intestinal mucosa. Smears prepared from crushed nodules and stained with Giemsa's stain revealed large numbers of sickle-shaped, uninucleate bodies, similar to Rainey's corpuscles of the Sarcosporidia. Material from the mucous folds and pieces from the wall of the abomasum were collected for histopathological study. Mounted sections stained with haematoxylin-eosin revealed these cysts to be typical of *Globidium* infection.

Examination of a number of mounted sections showed that these cysts lay in between the cells of the mucosa of the abomasum. They had a diameter of 300–480μ and each was surrounded by a definite wall, the thickness of which ranged from 5 to 6μ. This wall had two clear zones: an inner zone 2–2·5μ thick, which had a concentric structure and stained homogeneously, and an external zone, which had a divergent perpendicular arrangement. The cysts were filled with a huge mass of spores, each of which was a banana-shaped cell with a nucleus. The spores were 4·5–7μ long by 1·2–1·4μ wide. The nucleus in some was near the broader end, as it is in the classical description of *G. gilruthi*, in others it was nearer to the hyaline, narrow end. In some sections there was, in a few cysts, a large body resembling a nucleus, which measured 26–30 by 65–76μ and some spores were attached to the outer part of this. Coccidial merozoites were not

* Present address, Research Division, Department of Animal Production, Ministry of Animal Resources, Khartoum, Sudan.

found in any of the sections examined. On the above evidence these cysts were diagnosed as cysts of *G. gilruthi* (Chatton, 1910).

A number of sheep and goats were examined in the Cairo abattoir to find out the percentage of infection with this parasite. The check was limited to examination of their abomasa. For this purpose 250 sheep and 150 goats were investigated during February, March, April, May and June, 1955. 18% of the sheep and 28% of the goats showed this protozoan infection. Triffit (1925) recorded an infection rate of 92% with *G. gilruthi* among sheep in Britain. Sarwar (1951) found that 34% of the sheep and goats slaughtered at Lahore abattoir were infested with *G. gilruthi*, and that in other localities of East Punjab as many as 94% were infested.

The percentage of infection here recorded is only the minimal infection rate, as it was carried out under conditions which allowed only quick inspection, and lighter infections may have escaped notice. Few cases were heavily infected and these were mainly among goats. Histopathological examination was carried out on some cases and confirmed the previous findings.

Wenyon (1926) stated that haemorrhages may occur as a result of rupture of mature cysts in the abomasum. Richardson (1948) mentioned that no pathogenicity has been attributed to *G. gilruthi* In this study heavy infestations with *G. gilruthi* were seen in debilitated sheep and goats, but other associated factors, such as haemonchosis, were more probable causes of the debility of the hosts. Heavy infections were also found in animals that were in good condition. It is therefore necessary to investigate the possible pathogenicity of this organism before its clinical importance can be assessed.

SUMMARY

The occurrence of *Globidium gilruthi* in sheep and goats in Egypt is recorded. Preliminary investigation showed that 18% of 250 sheep and 28% of 150 goats were infected. The lesions caused by *G. gilruthi* are described.

REFERENCES

CHATTON, E. (1910). Le Kyste de Gilruth dans la muqueuse stomacale des ovidés. *Arch. Zool. exp. gén.* **45**, 55, vol. 5 (Notes et Rev. (4)), pp. 414–24.

RICHARDSON, U. F. (1948). *Veterinary protozoology*, 1st edn. p. 178, Oliver and Boyd, Edinburgh.

SARWAR, M. M. (1951). Occurrence of *Globidium Gilruthi*, A protozoon parasite of sheep and goats, from the Indo-Pakistan sub-Continent. *Parasitology*, **41**, 282.

TRIFFIT, M. J. (1925). Observations on *Gastrocystis gilruthi*, a parasite of sheep in Britain. *Protozoology*, **1**, 7–18.

WENYON, C. M. (1926). *Protozoology*, vol. 1, p. 772. London: Baillière, Tindall and Cox.

(*Manuscript received for publication* 15. v. 1957.—Ed.)

THE GAMETOGENESIS OF THE DIGENETIC TREMATODE *SPHAEROSTOMA BRAMAE* (MÜLLER) LÜHE

By R. A. R. GRESSON

Department of Zoology, The Queen's University of Belfast

(With Plates XI and XII)

INTRODUCTION

Although the germ-cells of a number of digenetic trematodes have been studied, many problems of gametogenesis await solution. For example, several workers claim that the spermatozoon is wholly nuclear in origin, while a minority believe that the cytoplasm of the spermatid contributes to its formation. The present investigation was undertaken primarily with the object of determining the origin and structure of the spermatozoon. As the testes of *Sphaerostoma bramae* were found to be favourable material for study, it was decided to investigate not only the stages of spermateleosis, but to give a brief account of the process of spermatogenesis. In addition, the structure of the primary oocyte and the behaviour of the Golgi elements and mitochondria of the germ-cells were studied.

Most previous workers are agreed that the primary spermatogonium of digenetic trematodes divides to give two secondary cells, and that the latter divide to give a group of four tertiary spermatogonia. The primary and secondary spermatocytes and the spermatids are present in groups of eight, sixteen and thirty-two cells respectively. There appears to be some variation in the relationship between the cells that constitute a group. In some cases, it is claimed, the protoplasm of the inner pointed ends of the male germ-cells remain continuous throughout certain phases of spermatogenesis, while in other trematodes the cells become completely separated after each division. Yosufzai (1952*a*) claimed that in *Fasciola hepatica* the primary and secondary spermatocytes and the spermatids are attached to a central disk or blastophore composed of cytoplasm, 'and possibly some nuclear material', which is cut off from their inner ends when the primary spermatocytes are formed. He considered it probable that this structure is similar to the hollow central ball recorded by Markell (1943) and to the cyncytium observed by Cable (1931) in the testis of *Probilotrema californiense* and *Cryptocotyle lingua* respectively.

Few attempts have been made to study the cytoplasmic inclusions of the germ-cells of trematodes. Yosufzai's (1952*a*, *b*) accounts of his work on *Fasciola hepatica* are the most complete records of this aspect of gametogenesis in these animals. Dingler (1910), who observed mitochondria in a single spermatogonium and in the spermatids of *Dicrocoelium lanceolatum*, appears to have been the first to note the presence of mitochondria in a trematode. Later, Severinghaus (1927) stated that 'rings of chondriosomal material of varying sizes' are present in the late spermatids of *Schistosoma japonicum* where they take part in the formation of the tail sheath of the spermatozoon. No further information is given regarding

their behaviour. This worker does not appear to have studied the mitochondria of the female germ-cells.

Yosufzai (1952*a*, *b*) recorded for the first time the occurrence of Golgi elements in the germ cells of a trematode. More recently, Dhingra followed the history of the Golgi elements and the mitochondria in the male germ-cells of *Isoparorchis eurytremum* (1954*a*). According to him, while mitochondria are clearly visible, Golgi bodies are absent from the male germ-cells of *Cotylophoron elongatum* (1955*a*) and of *Gastrothylax crumenifer* (1955*b*).

MATERIAL AND METHODS

Specimens of *Sphaerostoma bramae* (Müller) Lühe were obtained from the intestine of bream, *Abramis brama* (L.). This trematode was first identified as occurring in Ireland by Miss M. Pauline Corbett, M.Sc., while working in this department (to be published). Bream were netted by a local fisherman in Lough Erne, Co. Fermanagh, during spring, late summer and autumn. The alimentary canal was removed and the trematodes recovered on the day following the capture of the fish. In most cases the parasites were alive when removed from the intestine; living specimens were retained and fixed for cytological purposes.

For the study of the structure of the gonads and the general features of gametogenesis, specimens were fixed in Carnoy and Bouin's picro-formol. The latter proved to be the more satisfactory, and consequently was used throughout the investigation. Sections were cut at 5 and 7·5μ in thickness and subsequently stained in iron haematoxylin. In some cases eosin or light green was used as a counterstain. Material for the study of the nucleus of the late spermatid and the spermatozoon was treated according to the Feulgen technique. Sections were cut at 7·5μ in thickness.

The Golgi elements were studied in material treated according to the method of Kolatchev, and the mitochondria in gonads fixed in Flemming, and in Kolatchev preparations. Sections were cut at 5μ in thickness.

OBSERVATIONS

Spermatogenesis

Spermatogonia. Sections of the testes of mature specimens frequently contain cells at all stages of spermatogenesis, including numerous late spermatids and spermatozoa. The primary spermatogonia are few in number and are usually arranged in a single layer at intervals along the outer wall of the testes, or less often in isolated groups three or four cells deep. In some few cases, however, they form a continuous layer extending for some distance round the testis. Each primary spermatogonium possesses a relatively large nucleus with a single, deeply stained nucleolus. The majority of cells are round or oval in outline, but there is considerable variation in their shape.

The secondary spermatogonia are not numerous in the material examined. They are somewhat elongate cells and are present in pairs or else in small groups. They may be in contact with the primary spermatogonia or with the wall of the testis.

The tertiary spermatogonia are similar in appearance to the secondary cells but are not as large. They tend to occur in groups of four; frequently, however, owing to the close proximity of several clumps, grouping is not obvious. Tertiary spermatogonia are often in contact with the wall of the testis. Careful examination of secondary and tertiary spermatogonia failed to reveal the presence of protoplasmic connexions between neighbouring cells.

In a few cases primary spermatogonia in the early prophase were visible, but no other stages of mitosis were observed. The absence of such stages was due in all probability to the advanced condition of the testes of most of the individuals recovered during the present investigation.

Primary spermatocytes. The resting primary spermatocytes are large cells each provided with a relatively large nucleus containing numerous small granules and a centrally placed nucleolus that stains deeply with haematoxylin. They occur in groups of eight, which are often most numerous in the peripheral regions, where they are sometimes in contact with the wall of the testis. Each cell possesses a narrow region directed towards the centre of the clump. A careful examination of these narrow central regions showed that protoplasmic connexions between adjacent spermatocytes or a central blastophore are lacking. In section, the shape of the cells and the manner in which they are arranged in a group are not always apparent.

With the onset of the prophase of the first meiotic division, the chromatic material of the nucleus comes to occupy a central position. Later, this material forms a dense clump close to the nuclear membrane. As the prophase proceeds, the clump becomes less dense and thread-like chromosomes make their appearance and fill the greater part of the nucleus. At the same time the nucleolus again becomes visible (Pl. XI, figs. 1, 5). Many of the sections examined contain primary spermatocytes in metaphase and anaphase (Pl. XI, fig. 2). Counts indicate that twelve bivalent chromosomes are present.

Secondary spermatocytes. Secondary spermatocytes are present in groups of sixteen and may occur in any part of the testes, except the extreme peripheral region. These cells and their nuclei are smaller and stain more faintly with haematoxylin than the primary spermatocytes. A single small nucleolus is present. The narrow end of each cell is directed towards the centre of the group, but, as in the case of the primary spermatocytes, the shape and disposition of the cells are not always evident in sections. A central blastophore and protoplasmic strands appear to be absent. Secondary spermatocytes in prophase, metaphase, anaphase and telophase are often visible in many of the sections through a testis. Owing to the small size and clumping of the chromosomes, it is difficult to make an accurate determination of their number, but examination of metaphase plates indicates that the diploid number is twenty-four.

Spermateleosis. The young spermatids occur in groups of thirty-two, which are often packed closely together and may be in juxtaposition to primary or secondary spermatocytes. Consequently, the grouping of the cells is often obscured. They do not appear to be provided with marked centrally directed, pointed regions similar to those of the primary and secondary spermatocytes. A central blastophore is absent.

The young spermatids are oval or slightly elongate and possess round or oval nuclei. In preparations stained with haematoxylin, each nucleus contains a coarse network of chromatic material. Later, the nucleus becomes fusiform (Pl. XI, fig. 3), and now stains deeply with the exception of a few small spaces in its interior. It continues to elongate and is transformed into a filamentous body which becomes bent and curved within the cell (Pl. XI, figs. 3, 4). At a later stage one end of the filament, which is very slender and is composed of deeply stained granules, extends beyond the inner somewhat pointed end of the cell (Pl. XI, fig. 6). During the elongation of the nucleus the cell increases somewhat in size and the cytoplasm becomes vacuolated and loses much of its affinity for stains. Later, the filament becomes free of the vacuolated cytoplasm of the spermatid and forms an elongate, deeply stained thread-like structure which, I believe, is the nucleus of the future spermatozoon (Pl. XII, fig. 7).

The nucleus of the sperm is at first somewhat granular, but later decreases slightly in diameter and becomes more compact in appearance. It is slightly pointed at one end, and at the opposite, or posterior, end is attached to a lightly stained tail or flagellum, which is somewhat longer than the nucleus. In order to determine with a greater degree of certainty the area of the nucleus and whether, in fact, cytoplasmic material is included in the spermatozoon, sections were treated according to the Feulgen method. In these sections the nucleus gives a strong positive reaction, while the more slender terminal region of the sperm is negative and, when light green is used as a counter-stain, it is coloured by this dye. A study of late spermatids present in Feulgen preparations yielded further evidence in favour of the nuclear origin of the entire anterior region of the spermatozoon. It was not possible to follow the development of the sperm in greater detail than that described above. The transformation of the nucleus of the spermatid into the elongate nucleus of the spermatozoon was clearly shown in a great number of the sections examined. The origin of the tail region, however, could not be followed with certainty. Presumably a small amount of cytoplasm is liberated together with the nucleus and becomes organized into a long flagellum. The greater part of the extranuclear region of the spermatid, however, remains as a residual mass which undergoes degeneration. Attempts to elucidate the internal structure of the tail were unsuccessful, and a centriole was not identified in either the spermatid or the spermatozoon. An external limiting layer was not visible around the nucleus of the sperm. It is reasonable to suppose that such a layer or membrane exists, but is so slender that it could not be detected by the methods employed during the present investigation.

Golgi elements and mitochondria. Golgi elements were observed in cells at all stages of spermatogenesis, except during the metaphase and later phases of mitosis. In the spermatogonia they are situated at one pole of the nucleus and are often collected into a fairly compact mass. In other cases the Golgi elements are more widely separated and are clearly visible as short filaments and as rods which surround a material which is very faintly blackened by osmium tetroxide. In deeply impregnated preparations small granules are frequently visible scattered through the cytoplasm. These are probably mitochondria. An examination of testes fixed in

Flemming and stained with acid fuchsin showed that large, granular mitochondria are distributed through the cytoplasm. In some cases the granules are most numerous at one pole of the nucleus.

In the early primary spermatocytes the Golgi elements are closely packed together at one pole of the nucleus (Pl. XII, fig. 8). Later, the Golgi rods and filaments are more widely separated and appear to be moving out into the surrounding cytoplasm. Although stages of meiosis were not observed in Kolatchev preparations, it is probable that these changes are preparatory to the distribution of the Golgi elements throughout the cytoplasm, prior to the division of the cell. In Flemming preparations, mitochondria, in the form of granules and short rods, are more numerous than in the spermatogonia. They are frequently scattered fairly evenly through the cell, but in some cases are concentrated chiefly between the nucleus and the inner pointed end of the cell. In this region granules are often in alignment, so that they resemble filaments. Small osmiophil granules seen in some of the Kolatchev material are probably mitochondria.

In young spermatids the Golgi elements are situated at one pole of the nucleus. They are in the form of filaments and rod-shaped bodies surrounding a faintly osmiophil substance. The Golgi bodies are not closely packed together, and therefore can be observed with greater ease than those of the spermatogonia and spermatocytes. Examination of cells in which the nucleus is undergoing elongation reveals a loose clump of Golgi elements lying in the cytoplasm some little distance from the nucleus. A study of these cells indicates that the Golgi bodies increase and finally break up to form a number of deeply osmiophil, short rods and rounded bodies. They remain in the residual cytoplasm and apparently degenerate.

Granular mitochondria are visible in many of the older spermatids. They are not numerous and remain in the residual cytoplasm.

Oogenesis

Primary oocytes. The ovary of a mature individual is usually completely filled with primary oocytes, but in some cases a few small cells are situated at its periphery; it is probable that the latter are oogonia. The primary oocytes are large cells closely packed together. Their nuclei may be in the resting condition or in the very early prophase of the first meiotic division.

The non-dividing cells are large and possess round or oval nuclei. A single, large, deeply stained nucleolus with a more lightly stained central region is present. Chromatic granules and faintly stained threads are situated between the nucleolus and the nuclear membrane. As the nucleus passes into the prophase, the nucleolus decreases somewhat in size and its central area stains less deeply than during the resting stage. Both the nucleus and the nucleolus of cells in the leptotene stage are smaller than those of cells in the preceding phases. The nucleolus now stains homogeneously and the chromosome threads are polarized. The pairing of the zygotene threads and of the chromomeres may readily be observed in favourable preparations.

As stages later than the prophase of the first maturation division were not observed, it is probable that this process is completed after the egg has left the ovary. Few of the individuals sectioned during the present investigation contained

uterine eggs. In these specimens the number of eggs was small and owing to the hardness of the shell sections were unsatisfactory for cytological examination. Consequently, fertilization and cleavage were not investigated.

In primary oocytes which have been deeply stained with haematoxylin several small granules are scattered through the nucleoplasm, some of which are in contact with the inside of the nuclear membrane. Bodies similar in size and in staining properties occur in the cytoplasm. It is probable, therefore, that nuclear material is passed into the cytoplasm where it is visible in the form of granules.

Golgi elements and mitochondria. The Golgi elements of the young primary oocytes are often collected into a compact mass situated close to, or in contact with, the nuclear membrane (Pl. XII, fig. 9). In later cells this mass is less compact and is seen to be composed of short, rod-like Golgi elements. These Golgi bodies move from their original position and presumably at a later stage are distributed through the cell. In some oocytes two or three groups of Golgi elements are present (Pl. XII, figs. 10, 12). It would appear, therefore, that the single clump of Golgi bodies may separate into two or three parts before the elements migrate through the cytoplasm.

Mitochondria are clearly visible in Flemming preparations stained with acid fuchsin. They are in the form of granules, some of which are in alignment, and of short rods. The majority are often concentrated in a cap-like or crescent-shaped mass at one pole of the nucleus, or else form two clumps lying at opposite poles of the cell. In some cases a concentrated mass of mitochondria may almost completely surround the nucleus. It is probable that this condition represents a stage in the dispersal of the mitochondria throughout the cytoplasm (Pl. XII, fig. 11). In addition to those in the clumps, a few mitochondria are scattered through the cell.

DISCUSSION

In general the stages of gametogenesis of *Sphaerostoma bramae* follow the usual pattern of this process in the Digenea. References to much of the literature of the subject will be found in some of the works cited in the present paper and need not be given here.

It has been claimed that the male germ-cells of some digenetic trematodes remain connected centrally subsequent to certain of the nuclear divisions that result in their formation. Such claims have been made by Chen (1937) for *Paragonimus kellicotti*, Dingler (1910), for *Dicrocoelium lanceolatum*, Dhingra for *Isoparorchis eurytremum* (1954*a*), *Cotylophoron elongatum* (1955*a*) and *Asymphylodora* sp. (1955*c*), and Rees (1939) for *Parorchis acanthus*. Careful studies of all stages of the spermatogenesis of *S. bramae* failed to reveal the presence of protoplasmic strands between neighbouring cells, or of a structure resembling the blastophore observed by Yosufzai (1952*a*) in the testis of *Fasciola hepatica*.

Yosufzai (1952*b*) found that nucleolar material is extruded to the cytoplasm of the oogonia and oocytes of *F. hepatica*. He believes that bodies previously seen by other workers in the ooplasm of trematodes were nucleolar in origin, but were not recognized as such. In view of Yosufzai's comments it is of interest that the extrusion of nuclear material was observed in oocytes of *Sphaerostoma bramae*. The

process, however, was not so marked as in *Fasciola hepatica*. The passage of material from the nucleus of female germ-cells has been recorded for a number of animals of other phyla. Recently, Anderson & Beams (1956) have demonstrated by means of electron microscopy that the nuclear membrane of the nurse-cells of the bug, *Rhodnius prolixus*, possesses pores and that granular nuclear material passes through these pores to the cytoplasm.

In *Sphaerostoma bramae* the Golgi elements, as revealed in Kolatchev preparations, are in the form of rods and short filaments which, in the young spermatogonia, spermatocytes and spermatids, are localized at one pole of the nucleus. Although stages of mitosis or meiosis were not observed in osmic preparations, it is probable that the subsequent movement of the Golgi bodies from the localized condition results in their distribution preparatory to cell division. In *Fasciola hepatica* a single clump of Golgi bodies is present in the primary and in the young secondary spermatogonia. The elements of the older secondary cells separate to form two groups and finally three to five clumps. Similar groups are present in the tertiary spermatogonia, spermatocytes and spermatids. Multiple groups comparable to these of *F. hepatica* were not seen in the male germ-cells of *Sphaerostoma bramae*.

According to Dhingra (1954*a*) a single Golgi body in the form of a 'minute granule' is present in the tertiary spermatogonium of *Isoparorchis eurytremum*. In the primary and secondary spermatocytes a similar small granule is at first present, but later it is converted into a body with a deeply impregnated cortex and a lightly impregnated central area. During the divisions of the spermatocytes the Golgi body separates into two parts, and each of the resulting 'granules' moves towards an opposite pole of the spindle. The 'Golgi granule' of the young spermatid increases in size, differentiates into a cortical and an inner region and finally decreases in size and disappears. It does not, therefore, take part in the formation of the spermatozoon.

The male germ-cells of both *Sphaerostoma bramae* and *Fasciola hepatica* possess numerous Golgi elements. The Golgi bodies of some of the spermatogonia and spermatocytes and of the spermatids of *Sphaerostoma bramae* are arranged around a material which is faintly blackened by osmium tetroxide. It is possible that a similar arrangement exists in *Isoparorchis eurytremum* and that this condition was interpreted by Dhingra as representing a single body consisting of a cortex and a central region. In very deeply impregnated material the Golgi clumps of *Sphaerostoma bramae* might be mistaken for single bodies.

Yosufzai was unable to follow the history of the Golgi elements in the late spermatid of *Fasciola hepatica*. He concludes that, as there is no evidence that cytoplasm is eliminated from the spermatid, 'it is probable that Golgi bodies are included in the spermatozoon'. In *Sphaerostoma bramae* the Golgi elements remain in the residual cytoplasm. This agrees with Dhingra's observation that the Golgi bodies of *Isoparorchis eurytremum* take no part in the formation of the sperm.

It would appear that the behaviour of the Golgi bodies of the primary oocytes of *Sphaerostoma bramae* is somewhat similar to those of the oocytes of *Fasciola hepatica*. Yosufzai, however, found that the Golgi elements, in the form of granules and short

rods, spread out from a juxtanuclear mass in the young oocyte and that the majority come together again to form a compact clump at one pole of the nucleus or else two masses at opposite sides of the nucleus. In the older oocytes granular Golgi bodies are present throughout the cytoplasm. In *Sphaerostoma bramae* complete dispersal of the Golgi rods was not observed. It would seem, therefore, that in this species dispersal does not take place until after the oocyte leaves the ovary.

Work with the electron microscope indicates that the Golgi elements of the cells of invertebrate animals resemble those of the cells of vertebrates. In the latter the Golgi complex is composed of membrane systems frequently associated with vacuoles, granules and a ground substance (Sjöstrand, 1956). Dalton & Felix (1956) have shown that the Golgi complex exhibits constant characteristic features in a variety of cell types. It would seem, therefore, that the Golgi elements seen in osmic preparations of the germ-cells of *S. bramae* represent Golgi membrane systems probably associated with vacuoles and minute granules or vesicles. Vacuoles, if present, are presumably very small and are not visible under the light microscope. I have pointed out elsewhere (Gresson, 1957) that, if the Golgi element is a membrane system, then such structures should not be called granules. In Kolatchev preparations of the germ-cells of *S. bramae*, osmium is apparently deposited on or around the membranes. Dalton & Felix (1956) find that osmium is not reduced in the Golgi membranes but in the small vesicles or vacuoles, and, in the case of the epididymis, between the membranes and within the large vacuoles.

In *Fasciola hepatica*, according to Yosufzai, mitochondria surround the nucleus of the late spermatid, while others are included in the middle-piece of the sperm. Dhingra, on the other hand, believes that the mitochondria of the spermatids of *Isoparorchis eurytremum*, *Cotylophoron elongatum* and *Gastrothylax crumenifer* remain in the residual cytoplasm. That those of *Sphaerostoma bramae* do not take part in the formation of the spermatozoon is in agreement with Dhingra's findings for three other species of digenetic trematodes.

Yosufzai refers to previous work on the formation of the spermatozoon of trematodes and shows that, while several workers believe that the sperm is wholly nuclear in origin, others claim that the cytoplasm of the spermatid enters into its composition. He believes that in *Fasciola hepatica* the sperm consists of a definite head and tail and that the latter is cytoplasmic in origin. More recently, Dhingra claims that the spermatozoon of *Isoparorchis eurytremum* is provided with two flagella and that these arise from the centrosome of the spermatid. According to him the single flagellum of the spermatozoa of *Cyclocoelium bivesiculatum* (1954*b*), *Cotylophoron elongatum* (1955*a*) and *Gastrothylax crumenifer* (1955*b*) also arises from a centrosome present in the late spermatids. In these animals the sperm is composed of a nucleus, a centrosome and a flagellum.

Careful examination of the spermatids and spermatozoa of *Sphaerostoma bramae* failed to reveal the presence of a centrosome. The elongate nucleus of the sperm is Feulgen-positive, thus confirming conclusions resulting from the study of testes stained with haematoxylin. It is concluded that the spermatozoon of this trematode is nuclear and cytoplasmic in origin and that the nucleus may be surrounded by a very thin limiting layer of cytoplasm.

SUMMARY

The stages of spermatogenesis and the structure of the primary oocyte of *Sphaerostoma bramae* were studied in material fixed in Bouin and Flemming's fluid and in preparations treated according to the Kolatchev and the Feulgen techniques.

Chromosome counts of primary and secondary spermatocytes indicate that the diploid number is twenty-four.

The stages of spermatogenesis, in general, conform to the usual pattern of this process in digenetic trematodes. The spermatogonia, spermatocytes and early spermatids are not connected together by central strands, nor by a central disk, as is claimed for some other species. A study of sections stained in haematoxylin and of Feulgen preparations showed that the spermatozoon is composed of an elongate nucleus and a tail. It was not possible, with the methods employed during the present investigation, to determine the fine structure of the tail.

The primary ovarian oocytes are in the interphase or in early prophase of the first maturation division. There is evidence that material is extruded from the nucleus to the cytoplasm.

The Golgi elements of the male germ-cells are revealed in Kolatchev preparations as short rods and filaments. The Golgi elements of the spermatid are eliminated in the residual cytoplasm. Mitochondria, in the form of granular bodies and short rods, were visible in spermatogonia, spermatocytes and spermatids. Those of the spermatid remain in the residual cytoplasm.

Short, rod-like Golgi bodies are present in the primary oocytes. In the young cells they form a compact mass situated close to or in contact with the nuclear membrane. Later, the elements spread out through the cell. Granular and rod-shaped mitochondria are concentrated in a single mass at one pole of the nucleus or in two masses at opposite sides of the nucleus. A few mitochondria are scattered through the cytoplasm.

I have much pleasure in expressing my thanks to Miss Noelle E. Stinson, M.Sc., and Miss Patricia A. Hale, B.Sc., research assistants, for fixing, sectioning and staining most of the material, and to Mr Wm. Ferguson for taking the photomicrographs.

REFERENCES

Anderson, E. & Beams, H. W. (1956). Evidence from electron micrographs for the passage of material through pores of the nuclear membrane. *J. Biophys. Biochem. Cytol.* (suppl.), **2**, 439.

Cable, R. M. (1931). Studies on the germ-cell cycle of *Cryptocotyle lingua* Creplin. 1. Gametogenesis in the adult. *Quart. J. Micr. Sci.* **74**, 563.

Chen, P. D. (1937). The germ cell cycle in the trematode *Parogonimus kellicotti* Ward. *Trans. Amer. Micr. Soc.* **56**, 208.

Dalton, A. J. & Felix, M. D. (1956). A comparative study of the Golgi complex. *J. Biophys. Biochem. Cytol.* (suppl.), **2**, 79.

Lingler, M. (1910). Über die Spermatogenese des *Dicrocoelium lanceolatum*. *Arch. Zellforsch.* **4**, 672.

Dhingra, O. P. (1954*a*). Gametogenesis and fertilization in *Isoparorchis eurytremum*. *Res. Bull. Panjab Univ.* **44**, 21.

DHINGRA, O. P. (1954*b*). Spermatogenesis of a digenetic trematode, *Cyclocoelium bivesiculatum*. *Res. Bull. Panjab Univ.* **61**, 159.

DHINGRA, O. P. (1955*a*). Spermatogenesis of a digenetic trematode *Cotylophoron elongatum*. *Res. Bull. Panjab Univ.* **64**, 1.

DHINGRA, O. P. (1955*b*). Spermatogenesis of a digenetic trematode *Gastrothylax crumenifer*. *Res. Bull. Panjab Univ.* **65**, 11.

DHINGRA, O. P. (1955*c*). Gametogenesis, fertilization and cleavage in *Asymphylodora* sp. *Res. Bull. Panjab Univ.* **66**, 19.

GRESSON, R. A. R. (1957). The Golgi complex of the male germ-cells of mammals. *Cellule*, **58**, 249.

MARKELL, E. K. (1943). Gametogenesis and egg-shell formation in *Probilotrema californiense* Stunkard. *Trans. Amer. Micr. Soc.* **62**, 27.

REES, G. (1939). Studies on the germ cell cycle of the digenetic trematode *Parorchis acanthus* Nicoll. Part 1. Anatomy of the genitalia and gametogenesis in the adult. *Parasitology*, **31**, 417.

SEVERINGHAUS, A. E. (1927). Sex studies in *Schistosoma japonicum*. *Quart. J. Micr. Sci.* **71**, 653.

SJÖSTRAND, F. S. (1956). The ultrastructure of cells as revealed by the electron microscope. *Int. Rev. Cytol.* **5**, 455.

YOSUFZAI, H. K. (1952*a*). Cytological studies on the spermatogenesis of *Fasciola hepatica* L. *Cellule*, **60**, 7.

YOSUFZAI, H. K. (1952*b*). Cytological studies on the oogenesis of *Fasciola hepatica* L. *Cellule*, **60**, 167.

EXPLANATION OF PLATES XI AND XII

PLATE XI

Fig. 1. Primary spermatocytes. Interphase and early prophase. A few spermatogonia are shown at the top of the photomicrograph. Bouin. Haematoxylin, scale *a*.

Fig. 2. Primary spermatocytes. To show chromosomes. Bouin. Haematoxylin; scale *d*.

Fig. 3. Part of a group of spermatids to show elongation of nuclei. Bouin. Haematoxylin; scale *d*.

Fig. 4. Groups of spermatids to show stages of spermateleosis. Bouin. Haematoxylin and eosin; scale *b*.

Fig. 5. Part of Fig. 1 at a higher magnification; scale *c*.

Fig. 6. Part of group of spermatids to show a later stage of spermateleosis. Bouin. Haematoxylin; scale *d*.

PLATE XII

Fig. 7. Groups of sperms recently liberated from remains of spermatids. Bouin. Haematoxylin, scale *a*.

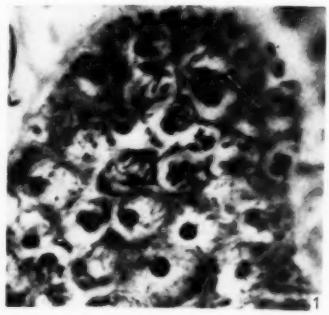
Fig. 8. Primary spermatocytes to show Golgi elements clumped at side of nucleus. Kolatchev, scale *a*.

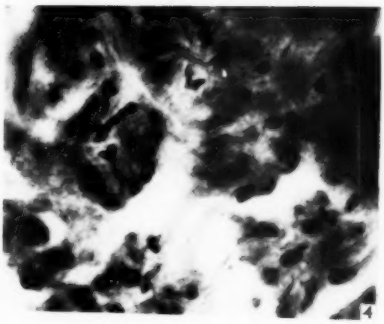
Figs. 9, 10. Primary oocytes to show Golgi elements. Kolatchev; scale *a*.

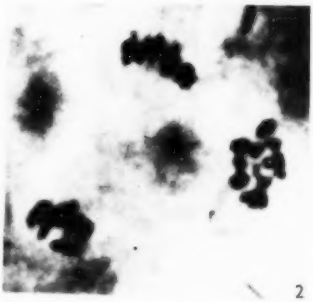
Fig. 11. Primary oocytes to show mitochondria. Flemming. Acid fuchsin; scale *b*.

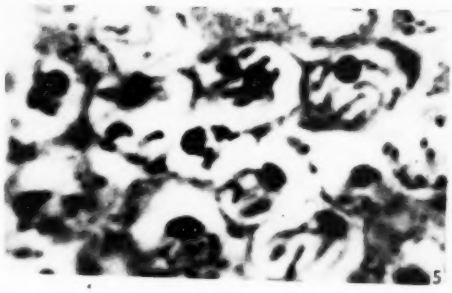
Fig. 12. Primary oocyte to show Golgi elements. Kolatchev; scale *a*.

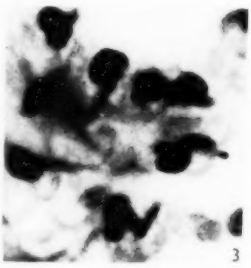
(*MS. received for publication* 2. VI. 1957.—Ed.)

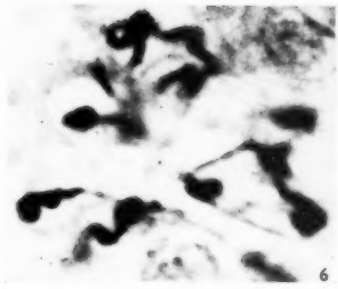












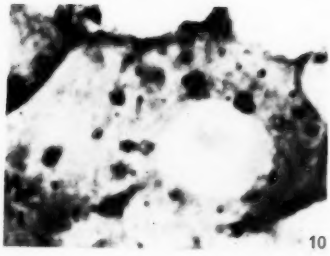
b
0·01 mm.

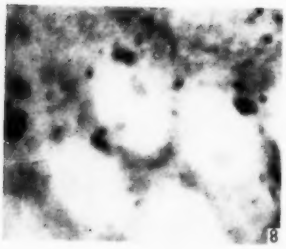
a
0·01 mm.

c
0·01 mm.

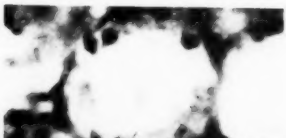
d
0·01 mm.

(*Facing p.* 302)





b
0·01 mm.



a
0·01 mm.

INFESTATION OF THE SLUGS *MILAX SOWERBII* FÉRUSSAC AND *AGRIOLIMAX RETICULATUS* MÜLLER BY TREMATODE METACERCARIAE (BRACHYLAEMIDAE)

By R. FOSTER. B.Sc., Ph.D.

*Zoology Department, The Durham Colleges in the University of Durham**

(With 2 Figures in the Text)

CONTENTS

		PAGE
I.	Introduction	303
II.	Geographical distribution of parasitism	303
III.	Seasonal fluctuations in the incidence of the infestation	305
IV.	Seasonal fluctuations in the incidence of the developmental stages of the parasites	307
V.	Observations concerning vertebrate hosts	308
VI.	Discussion	309
VII.	Summary	310
	References	311

I. INTRODUCTION

Unencysted brachylaemid metacercariae were discovered inhabiting the slugs *Milax sowerbii* Férussac and *Agriolimax reticulatus* Müller in Durham in 1949. Descriptions of the parasite and of its effects on its hosts have been reported previously (Cragg, Foster & Vincent, 1958; Foster, 1958), and the present paper deals with the local distribution, seasonal incidence and possible life cycle of the parasite.

Examinations were made of slugs from twelve localities in County Durham and also from Derbyshire, Oxfordshire and Surrey. Parasitization was found only in slugs from County Durham and, except for a few isolated individuals, all infested animals came from a three-quarter mile stretch of the bank of the River Wear in Durham City. This paper describes the ecology and distribution of parasitized slugs in the latter area during the period 1949–53.

It will be shown that the incidence of parasitism followed a regular pattern for some years, but that in 1953 it was suddenly and inexplicably reduced. Foster (1958) showed that parasitism by this brachylaemid caused a high mortality among the hosts, and it is an accepted ecological principle that parasitism is a factor in the regulation of the density of a species. The present observations suggest that such a factor may itself be removed suddenly from a population, and without obvious reason.

II. GEOGRAPHICAL DISTRIBUTION OF PARASITISM

Slugs were collected at nine stations along the river bank. Stations 1–3 and 9 were in open tree- or shrub-covered land with public access; stations 4–8 were in private gardens, 6 and 8 being relatively uncultivated. Stations 5 and 7 possessed

* Now with the Colonial Research Service, Tanganyika.

herbaceous borders, the others being kitchen gardens. Stations 4 and 5 were the only ones to be isolated from others by unbroken walls, etc. Stations 1 and 3 were well removed from human habitations, although open to public access; all other stations were close to houses.

It will be shown later that the incidence of infestation is not constant throughout the year, but that no sudden changes occur during the autumn. Table 1 shows the incidence of infestation in both host populations in the area during this period in 1952.

Table 1. *Incidence of infestation—autumn* 1952

	A. reticulatus		*M. sowerbii*	
Station	No. examined	Percentage infestation	No. examined	Percentage infestation
1	14	0	(1)	(100)
2	60	3·3	14	43·0
3	3	0	0	.
4	67	7·4	20	70·0
5	23	13·0	5	20·0
6	60	23·7	26	18·4
7	82	25·6	48	58·3
8	32	3·1	12	33·3
9	37	0	(2)	(0)

Table 2. *Comparative incidence of infestation (positive cases denoted by* +)

	Infestation recorded in		Incidence in *M. sowerbii* greater than in *A. reticulatus*	Incidence in *A. reticulatus* greater than in *M. sowerbii*
Area	*M. sowerbii*	*A. reticulatus*		
Station 1	+	.	.	.
Station 2	+	+	+	.
Station 4	+	+	+	.
Station 5	+	+	+	.
Station 6	+	+	.	+
Station 7	+	+	+	.
Station 8	+	+	+	.
Durham A	+	.	.	.
Durham B	+	.	.	.
Durham C	+	+	+	.

The infestation at station 1 appears to indicate that human habitations and their associated fauna are not necessarily involved in the life history of the parasite, and the presence of walls traversing the area had no apparent effect on the distribution of parasitism.

In the *Agriolimax reticulatus* population there appeared throughout the year to be a focus at station 7, the incidence of infestation decreasing as one proceeded away in either direction; among *Milax sowerbii* the incidence was more evenly distributed over the area. Further observations on the comparative incidence of

infestation are given in Table 2, which comprises data from all known brachylaemid infested localities. It is seen that *M. sowerbii* was parasitized more frequently than *A. reticulatus*, and that infested individuals of the former species could live alongside an *A. reticulatus* population which showed no evidence of parasitism. Further, in all areas where both species were parasitized, except at station 6, the incidence of infestation was higher in the *M. sowerbii* population. These observations lead to the conclusion that *M. sowerbii* is a natural intermediate host for the parasite and that the penetration of the latter into *A. reticulatus* is a secondary and unnatural phenomenon. This conclusion is supported by the laboratory finding that *M. sowerbii* tolerated the effects of the parasitism longer than did *A. reticulatus* (Foster, 1958).

Individuals of both host species of less than 150–200 mg. in weight were not parasitized.

III. SEASONAL FLUCTUATIONS IN THE INCIDENCE OF THE INFESTATION

In *A. reticulatus* metacercarial infestation occurred in spring, about March or April. The incidence of infestation rose rapidly, reaching 90–100 % in June and July. During midsummer the incidence dropped sharply owing to the death of many parasitized individuals and their replacement in the population by younger, healthy animals. The level of infestation during autumn and winter showed a slow, steady decline, minimum values occurring about February prior to the spring increase. A small rise in the incidence occurred during late autumn or winter because of a secondary autumnal infestation by metacercariae developed from adult parasites in the late summer (see later).

The comparative scarcity of *M. sowerbii* in the area has made the records incomplete, but data obtained showed that *M. sowerbii* also became infested during the spring, but that the incidence reached a peak during late summer and autumn and did not fall until the winter. This again reflects the greater tolerance of the parasitism by the natural host species (Foster, 1958). The incidence of infestation among *M. sowerbii* was consistently higher than among *A. reticulatus*. Data are given in Table 3 and Fig. 1.

The incidence of infestation is known to have followed the above pattern from October 1949 to February 1953. Widespread changes occurred, however, in the parasite density during the spring of 1953, as shown by the data given in Table 4. In this table the 1952 autumnal infestation is referred to as the 1952 secondary infestation, and the 1953 normal spring infestation as the 1953 primary infestation.

It is seen that during the spring no primary infestation was recorded in the *M. sowerbii* population and that only a low, temporary incidence in March occurred among the *A. reticulatus* population. By July, when the 1952 secondary infestations had died out, the entire population appeared to be free of parasitism. But, during the autumn, parasites reappeared in the *M. sowerbii* population, the incidence reaching a peak of 61 % in September and remaining fairly high until late November, when the customary winter decline occurred. In the *A. reticulatus* population

a primary infestation also appeared during the late summer, but the incidence of parasitism remained low throughout the period and did not show the usual seasonal variations. At the end of November a small 1953 secondary infestation was recorded among *M. sowerbii*, but not among *A. reticulatus*.

Table 3. *Seasonal incidence of infestation*

Month	Percentage infestation in *A. reticulatus*	Percentage infestation in *M. sowerbii*
Sept. 1951	38	63
Oct.	22	66
Nov.	25	71
Dec.	13	.
Jan. 1952	6	50
Feb.	5	57
Mar.	8	(100)
Apr.	45	27
May	42	60
June	95	.
July	97	.
Aug.	50	.
Sept.	48	.
Oct.	40	55
Nov.	33	62
Dec.	25	52
Jan. 1953	37	22
Feb.	27	0

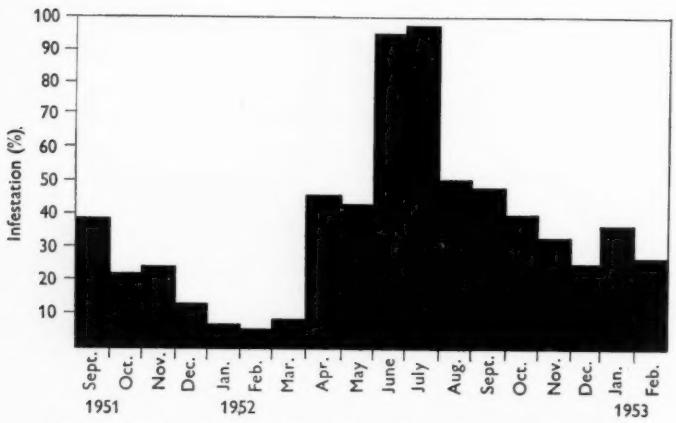


Fig. 1. *Agriolimax reticulatus*: seasonal incidence of infestation (September 1951–February 1953).

When metacercariae reappeared in August, they were of the later corrugated and smooth stages (Cragg *et al.* 1958) and the infestation appeared morphologically normal for the season. Because the earlier growth stages of the parasites had not been found during the spring and summer, the origin of the autumnal metacercariae is obscure.

Table 4. *Incidence of infestation during* 1953

Month	Percentage infestation in *A. reticulatus* 1952 Secondary	*A. reticulatus* 1953 Primary	*M. sowerbii* 1952 Secondary	*M. sowerbii* 1953 Primary
Jan.	37	0	22	0
Feb.	31	0	0	0
Mar.	25	6	27	0
Apr.	23	0	9	0
May	7	0	13	0
June	4	0	0	0
July	0	0	0	0
Aug.	.	17	.	17
Sept.	.	12	.	61
Oct.	.	20	.	40
1–15 Nov.	.	0	.	40
16–30 Nov.	.	9	.	21
Dec.	.	16	.	29

IV. SEASONAL FLUCTUATIONS IN THE INCIDENCE OF THE DEVELOPMENTAL STAGES OF THE PARASITES

There were four stages in the development of the metacercariae, designated successively caudal, feeding, corrugated and smooth (Cragg *et al.* 1958). Table 5 and Fig. 2 show the seasonal variations in the incidence of occurrence of the different developmental stages during 1951 and 1952. A very few hosts were found to be carrying more than one stage of the parasite simultaneously.

The spring rise in the incidence of infestation was caused by the appearance of

Table 5. *Seasonal fluctuations in incidence of growth stages*

Month	Percentage of parasitized hosts infested with Caudal stage	Feeding stage	Corrugated stage	Smooth stage
Sept. 1951	0	0	29	72
Oct.	0	0	33	67
Nov.	0	0	50	50
Dec.	9	0	45	45
Jan. 1952	0	5	30	70
Feb.	0	0	21	73
Mar.	75	10	15	0
Apr.	50	50	0	0
May	79	21	0	0
June	4	69	27	0
July	9	40	51	0
Aug.	0	4	32	64
Sept.	0	5	45	50
Oct.	0	0	50	50
Nov.	4	1	25	70
Dec.	1	5	20	75
Jan. 1953	0	0	20	80
Feb.	0	0	20	80

a large number of young caudal-stage metacercariae. These reached a peak in number in late March, dropped slightly during April and then rose to a maximum during May. There was then a rapid decline in numbers leading to the disappearance of the stage in August. The histograms in Fig. 2 show that the pattern was repeated at later dates by the succeeding developmental stages.

Fig. 2 shows a secondary (autumnal) infestation by caudal-stage larvae during November or December. This was followed, a month later, by a secondary peak

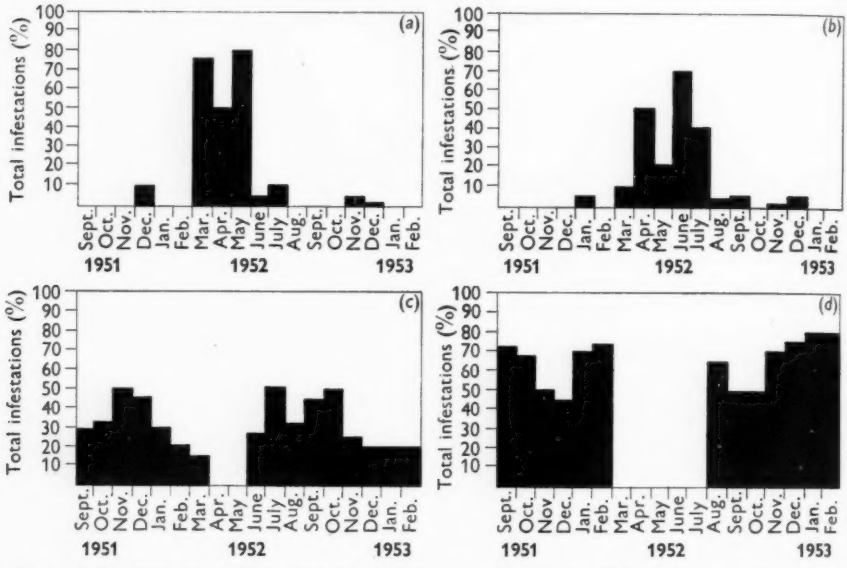


Fig. 2. *Agriolimax reticulatus*: seasonal incidence of developmental stages of metacercariae (September 1951–February 1953). *a*, caudal stage; *b*, feeding stage; *c*, corrugated stage; *d*, smooth stage.

of feeding-stage individuals. The gut contents of these parasites, however, remained as a loosely packed, greenish, non-crystalline mass and the cuticle showed only occasional corrugations. They increased very slightly in size and then remained unchanged, dying out the following spring and summer together with the primary corrugated and smooth stages of that year. It is possible that the unfavourable climatic conditions would cause these secondary infestations to die out without reaching maturity.

V. OBSERVATIONS CONCERNING VERTEBRATE HOSTS

Although no vertebrate host was found for this brachylaemid parasite, these ecological studies make it possible to postulate a life history of the species. All known Brachylaeminae utilize birds or mammals as the primary host. Considering the limited distribution of this species, it seems unlikely that a bird was involved, even allowing for the concept of territory. It seems more probable that a mammal was the vertebrate host and the terrain of the infested locality appeared suitable

for such animals as mice, voles and hedgehogs. The latter have been seen near to the infested area and these animals, together with voles and shrews, have been listed by previous authors (Stiles & Stanley, 1932; Dawes, 1946) as definitive hosts for brachylaemids, the former authors listing *Agriolimax* sp. as the intermediate host.

It is suggested, therefore, that the vertebrate host is a small mammal, possibly the hedgehog, which eats slugs avidly. This post becomes infested by smooth-stage metacercariae during autumn and early winter, the parasites maturing during the period of hibernation or decreased activity. When the mammal resumes full activity in the spring, brachylaemid eggs are passed in the faeces and ingested by browsing slugs, metacercariae appearing in March or April. Those appearing first will become smooth-stage forms in late summer and the mammal may acquire an infestation early enough to allow eggs to be passed before the onset of winter inactivity. Some slugs will thus acquire a larval infestation in the late autumn and this will account for the secondary metacercarial infestation of November or December.

This secondary infestation may itself indicate a likely vertebrate host. From the appearance of this infestation it is evident that only a short time elapses between the ingestion of larvae by the vertebrate and egg production by the adult fluke. Any suitable vertebrate which ingests larvae during the autumn will apparently carry mature adult flukes throughout the winter. The rodents are not true hibernators and, during a warm spell in the winter, they will forage for food. During such sorties trematode eggs, if they are present in the vertebrate's intestine, will be passed in the faeces, leading to instances of larval infestation among slugs during the winter. The fact that no such cases are known suggests that the vertebrate host is a true hibernator, which passes no eggs until it resumes full activity in the spring.

A further correlation between the known invertebrate host and the proposed vertebrate host lies in their nocturnal activity. If a bird were involved, many hours of possible desiccation could elapse between the passing of parasite eggs and their ingestion by a nocturnal slug. Conversely, in autumn, nocturnal vertebrates are more likely to encounter infested molluscs.

In the laboratory, mice, guinea-pigs, hedgehogs, and chickens were exposed to infestation by metacercariae, but all proved refractory. In the field, despite an extensive trapping programme, no animal was found to be carrying adult brachylaemids. But no hedgehogs could be obtained from the known infested locality, although they were known to be present near by. It may also be noted that the only hedgehogs obtainable for the laboratory work were heavily infested with nematodes (*Capillaria aerophila* and *Crenosoma* sp.), and this may have led to an immunity to further parasitism by trematodes.

VI. DISCUSSION

No host which harboured metacercariae also contained a sporocyst and the only sporocyst found (Cragg, *et al.* 1958) occurred in *Arion lusitanicus*, which does not normally harbour metacercariae. It seems likely that two intermediate hosts, which may or may not be of the same species, are involved, the sporocyst and cercariae inhabiting the first, and the metacercariae the second. Baer (1952) records

that brachylaemid cercariae may re-enter the first intermediate host via the urinary pore and may also penetrate other individuals in this manner. This could easily happen during coitus. Penetration into other species would be feasible via the ureter, when slugs are in contact during normal wanderings.

Although the infestation had lasted some years, parasitization declined sharply during 1953.

In the unnatural host, *A. reticulatus*, the maximum incidence of infestation during the year was 20 % in October. During the spring, no small mammals could be found in the area; if these animals were the definitive host of the parasite, their disappearance would explain the reduction in the parasite density. This theory is, however, improbable because a heavy infestation was later recorded among *M. sowerbii*. The climatic conditions in February and March were dry and windy and not favourable to great activity of either vertebrate or invertebrate hosts. But it is felt that these conditions would only have reduced contact between the host species to a small degree; they could have reduced the parasite density somewhat, but not to the observed extent. Further, *M. sowerbii* eventually showed a heavy infestation. As there was no decrease in the *A. reticulatus* population throughout the year the possibility that the parasite density was dependent on the population density of the host is apparently eliminated.

In the natural host species, *M. sowerbii*, no primary infestation was recorded until August, after which the incidence was comparable with that of previous years. It is not known why the younger stages of the metacercariae were not encountered before August, as presumably they must have been present to produce the corrugated and smooth stages seen during the autumn.

It is interesting to note, however, that, whatever changes actually occurred during the spring of 1953, the parasite recovered to a greater extent in the natural host population than among the unnatural hosts.

VII. SUMMARY

1. The parasitization of the slugs *Milax sowerbii* and *Agriolimax reticulatus* by a brachylaemid trematode in an area in Durham, during the period 1949–53, is described. Evidence is given that *M. sowerbii* is a natural intermediate host of the parasite.

2. Seasonal fluctuations in the incidence of infestation are described, including the fluctuations in the incidence of the four developmental stages of the metacercariae. Primary (spring) and secondary (autumnal) infestations of the molluscs are described.

3. The implications of an unexplained decline in parasite density during 1953 are noted.

4. A life history for the parasite, involving a small mammal as the vertebrate host, is postulated.

The author wishes to thank Professor J. B. Cragg for valuable advice during the investigation and for his help in the preparation of this paper. The work was financed by the Agricultural Research Council.

REFERENCES

BAER, J. G. (1952). *Ecology of Animal Parasites.* Urbana: University of Illinois Press.

CRAGG, J. B., FOSTER, R. & VINCENT, M (1958). Larval trematodes from the slugs *Milax sowerbii*, *Agriolimax reticulatus*, and *Arion lusitanicus*. *Parasitology*, **47**, 396–404.

DAWES, B. (1946). *The Trematoda.* Cambridge University Press.

FOSTER, R. (1958). The effects of trematode metacercariae (Brachylaemidae) on the slugs *Milax sowerbii* and *Agriolimax reticulatus*. *Parasitology*, **48**, 261–8.

STILES, C. W. & STANLEY, S. S. (1932). Key-catalogue of parasites reported for Insectivora, and their possible public health importance. *Bull. Nat. Inst. Public Hlth*, **159**, 791.

(*MS. received for publication* 14. VI. 1957.—Ed.)

STUDIES ON THE MORPHOLOGY, BIOLOGY AND DEVELOPMENT OF A STRIGEID CERCARIA (*CERCARIA X* BAYLIS 1930)

By DAVID A. ERASMUS

Department of Zoology, University College, Cardiff

(With Plate XIII and 6 Figures in the Text)

CONTENTS

	PAGE
Introduction	312
Methods	313
The Sporocyst	313
Cercaria X	314
Further development of *Cercaria X*	317
The metacercaria—*Diplostomulum X*	321
Host-parasite relationships	325
Histochemical studies	327
Completion of the life cycle	328
Relationships	328
Summary	329
References	332

INTRODUCTION

Very few species of furcocercariae have been recorded from British fresh waters. Brown (1926, 1931) recorded six new species (*Cercaria echinomorpha*, *C. chromatophora*, *C. pygocytophora*, *C. fissicauda*, *C. macrosoma* and *C. micromorpha*) from Cheshire. Three species (*Cercaria A*, *Szidat*, *Cercaria F.*1 and *Cercaria F.*2) have been described from Scotland by Harper (1931) and one unnamed species by Hesse (1932). Rees (1932) has described three species (*C. ocellata* La Valette, *Cercaria Y* and *Cercaria X* Baylis) from South Wales. *Cercaria F.*1 develops into a tetracotyle and *Cercaria X* into a diplostomulum metacercaria. The adult stage of *Cercaria A* is *Cotylurus cornutus* and that of *Cercaria ocellata* is said to be *Trichobilharzia ocellata*. Nothing is known of the further development of the other species.

Cercaria X was originally described in 1932 from Roath Park Lake, Cardiff and it is interesting to note that the species is still abundant in its original location. At the time of the original description specimens of *Lymnaea stagnalis* infected with *Cercaria X* were sent to Baylis and Taylor and they demonstrated experimentally (1930) that the cercariae penetrated fish and developed within the lens of the eye into diplostomula. Unfortunately, few details of development were given and their description of the metacercaria was based on immature specimens and was thus unsatisfactory for comparison with other described species.

The material presented in this paper represents a series of studies on *Cercaria X*, which produced further details of its development and enabled the structure of the fully developed metacercaria to be ascertained. In addition to the morphological studies, various aspects of the biology of the cercaria have been investigated.

METHODS

The majority of the morphological observations were made on fresh, living material and interpretation of details was facilitated by the use of coverslip pressure and the intravitam stain neutral red. Histological detail was investigated by using material fixed in Bouin and mercuric-acetic fixatives, and stained with either Heidenhain's or Weigert's haematoxylin and then counter-stained with Van Geison or Orange G. Great difficulty was experienced in sectioning the infected lens, and, after a number of unsuccessful attempts, the excessive hardening which occurred was avoided by dehydrating the material in the *n*-butyl-ethyl alcohol mixtures of Stiles (Bolles Lee, 1946), followed by double embedding according to the procedure of Peterfi (Carleton & Leach, 1938). The sites of lipase and phosphatase activity were determined by using material fixed in ice-cold acetone (Glick, 1949). Glycogen distribution was demonstrated by the techniques of Best, Bensley and Gomori (Glick, 1949), after fixation in Carnoy's fixative. Appropriate controls were included in all the histochemical tests.

Experimental infections with cercariae were achieved by immersing the host (fish and frog) in a suspension of newly emerged cercariae for a short period or by dropping a concentrated suspension on to the eye of rats. Infections with metacercariae were accomplished by feeding either entire eyes or isolated lenses to the experimental host. The faeces of experimental final hosts were collected daily and examined for eggs using the zinc sulphate flotation technique.

THE SPOROCYST

The first intermediate host in the life cycle is an aquatic snail. The molluscan fauna of Roath Park Lake is an extensive one and includes *Lymnaea stagnalis*, *L. pereger*, *Planorbis corneus*, *P. vortex*, *P. carinatus*, *Bithynia tentaculata* and the bivalves *Sphaerium corneum* and *Anodonta cygnaea*, but the sporocysts of *Cercaria X* have been obtained only from *Lymnaea stagnalis*. Although there has been no experimental confirmation of the fact, this restricted distribution suggests that the miracidium exhibits a rigid host specificity. Within the snail the sporocysts occurred mainly in the digestive gland, localizing in the connective tissue separating the tubules of the digestive epithelium. In very heavy infections the gland was almost entirely replaced by sporocysts.

The sporocysts are slender, unbranched, tubular structures and are pale cream in colour. They varied in length and one of the largest specimens obtained was 4 mm. long. In non-distended regions they were approximately 0·07 mm. in diameter. The sporocyst lumen decreases in diameter at one end, so that the animal terminates in a solid, conical mass of cells. This solid tip has at one side a well-defined birth-pore with protruding lips. The birth-pore is connected to the general lumen of the sporocyst by a narrow canal and this is capable of considerable distension allowing the passage of the cercariae to the exterior.

The sporocyst is completely covered by a cuticle which is approximately 0·001 mm. thick. As far as could be determined with standard techniques, the cuticle seemed to be devoid of spines and sculpturing. Below the cuticle are the

muscle fibres, divided into an outer layer of very fine, circular muscles and an inner layer of relatively much stouter, longitudinal fibres. The circular fibres run parallel to one another, but the longitudinal fibres run obliquely, crossing one another and forming a loose muscular network. The individual fibres are long and spindle-shaped. The sporocyst lumen is lined by an epithelium consisting of a single layer of cells, which possess large nuclei and a cytoplasm containing large, refractile yellow globules. The cream colour of the sporocyst is due to the presence of these yellow globules. The lumen of the sporocyst contains cercariae at all stages of development. Numerous large flame cells were observed, but attempts to elucidate a definite system were unsuccessful. Mature cercariae leave the sporocysts via the emergence canal and birth-pore, which contracts and closes up after the passage of each cercaria. In this way emergence of the cercariae does not necessitate the rupture and destruction of the sporocyst. In one dissected snail a large sporocyst was obtained from the region of the rectum. As this contained sporocysts and not cercariae it seems possible that at least two generations of sporocysts occur.

CERCARIA X

The cercaria is a typical pharyngeal, distome, longifurcous species (Text-fig. 1). Measurements of fixed and unfixed specimens are given in Table 1. The account given by Rees (1932) is correct in its general features and in the details of the excretory system, but the description of the spination was rather brief and further details are given below.

The oral cap is armed with a series of eighteen stout hooks which vary in size, the largest occurring in the mid-line of the ventral surface and the others decreasing in size towards the side of the body. Each hook (Text-fig. 2*a*) consists of a shaft bearing an arm and a recurved blade. The hook is orientated so that the shaft lies posteriorly, with the large blade projecting outwards and the smaller arm lying along the surface of the body. Posterior to the oral cap is a narrow spineless region enclosing the mouth and this is followed by a wide post-oral zone of hooks. This zone consists of seven to eight rows of hooks similar in structure to those of the pre-oral zone (Text-fig. 2*b*), but the shafts of these hooks are directed anteriorly and each hook is embedded in such a way that only the comparatively large, recurved blade protrudes from the surface. The hooks vary in size; those present in the anterior rows are larger than those in the posterior rows. This post-oral zone is followed by the general body spination consisting of an irregular pattern of short spines. At intervals along the body there occur rows of much larger spines and there is a total of eight such rows between the anterior organ and the ventral sucker. At the posterior end of the body project two fine hairs. The spination of the ventral sucker, tail stem and furcae is as described by Rees (1932).

A characteristic feature of the cercaria is the presence of refractile yellow globules, which are restricted to definite regions of the body. There is a concentration of these anterior to the ventral sucker and also at the posterior end of the body. The distribution of these globules coincides with the regions of glycogen deposition indicated by histochemical tests and it is possible that these refractile

globules represent the cells rich in glycogen. The caudal bodies, as will be described later, contain large amounts of glycogen and these also have a yellow refractile appearance. The free-swimming cercariae exhibit a characteristic orientation in the water and this is illustrated by Text-fig. 3.

Table 1. *Size of* Cercaria X

(*A*) Newly emerged specimens fixed in warm 10% formalin and measured in the fixative.

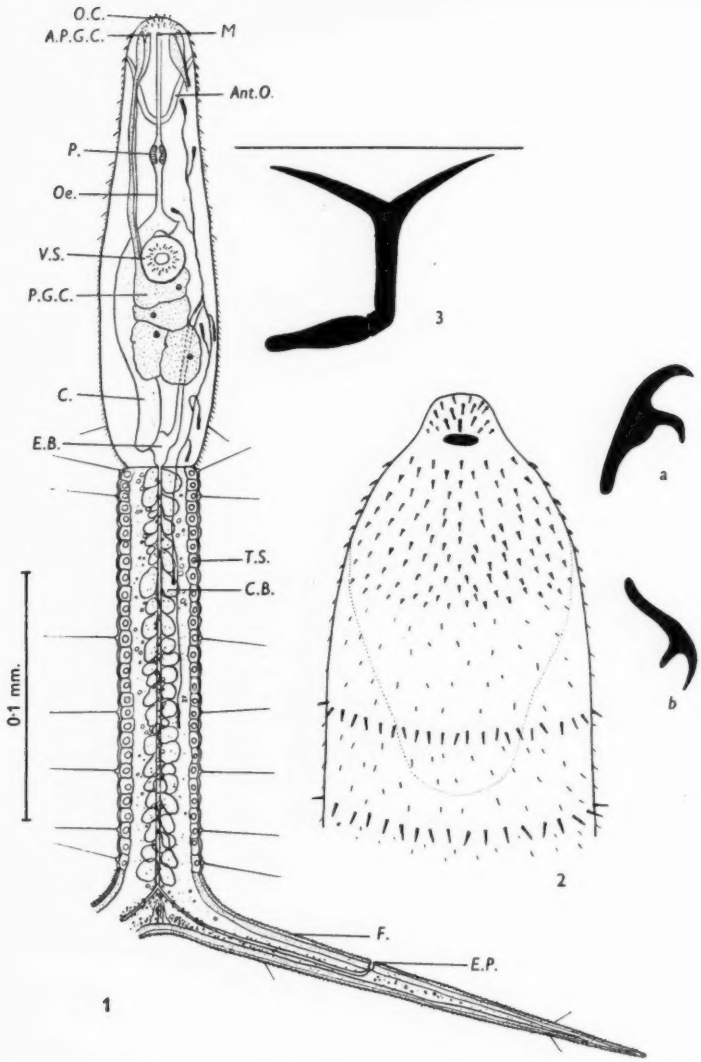
		Max. (mm.)	Min. (mm.)	Mean (mm.) (10 specimens)
Body	Length	0·126	0·135	0·185
	Width	0·048	0·038	0·041
Tail stem	Length	0·194	0·161	0·175
	Width	0·035	0·025	0·030
Furca	Length	0·225	0·177	0·198
	Width	0·022	0·012	0·015
Anterior organ	Length	0·045	0·048	0·047
	Width	0·029	0·022	0·024
Ventral sucker	Length	0·032	0·025	0·029
	Width	0·025	0·035	0·029

(*B*) Measurements (mm.) of heat-killed (unfixed) newly emerged specimens of *Cercaria X*.

		Max. (mm.)	Min. (mm.)	Mean (mm.) (10 specimens)
Body	Length	0·244	0·188	0·207
	Width	0·055	0·044	0·050
Tail stem	Length	0·233	0·220	0·226
	Width	0·033	0·033	0·033
Furca	Length	0·220	0·211	0·219
	Width	0·015	0·013	0·013
Anterior organ	Length	0·059	0·040	0·052
	Width	0·034	0·025	0·028
Ventral sucker	Length	0·034	0·027	0·032
	Width	0·034	0·029	0·032

Specimens were exposed to the heat of a 60 W. bench lamp for 30 sec. Care was taken to avoid evaporation and coverslip pressure whilst the measurements were being made.

As far as could be determined from the literature, *Cercaria X* has not been recorded from any other part of the world. However, it was found that two other species of cercariae (*C. chromatophora*, Brown, 1931. Great Britain; *C. chrysenterica*, Miller, 1926. North America) have been described, which possess the same flame-cell formula as *Cercaria X*. The characters of these two cercariae are compared with those of *Cercaria X* in Table 2. Of the three species *Cercaria X* and *C. chromatophora* are the most closely related and it seems probable that they are identical. *Cercaria X* is the earlier specific name and should be retained. *C. chrysenterica* differs in the number of caudal bodies in the tail-stem and, as this seems to be a constant character amongst cercariae of this type, specific differentiation may be maintained on that basis.



Text-fig. 1. Semi-diagrammatic view of the ventral surface of *Cercaria X*. Right furca, right side of excretory system and left intestinal caecum have been omitted from the diagram. Outline drawn from fixed material.

Text-fig. 2. Diagrammatic view of ventral surface of the anterior end of *Cercaria X* to show spination. 2*a*. Hook from oral cap. 2*b*. Hook from suboral zone. Not to scale.

Text-fig. 3. Diagrammatic representation of the floating position of *Cercaria X*.

Table 2. *A comparison of* Cercaria X, C. chrysenterica *and* C. chromatophora

Feature	*Cercaria X*	*C. chrysenterica*	*C. chromatophora*
Body size	0·207 × 0·05 mm.	0·26 × 0·048 mm.	0·27 × 0·05 mm.
Tail stem	0·226 × 0·033 mm.	0·244 mm.	0·23 mm.
Furca	0·219 × 0·013 mm.	0·248 mm.	0·25 mm.
Ventral sucker	0·032 × 0·032 mm.	0·045 mm.	0·03 mm.
Caudal bodies	25–30 pairs	Approx. 16	+, no. ?
Eye spots	.	.	.
Body hairs	1 pair	?	?
Tail-stem hairs	14	?	14
Penetration gland cells	4, posterior to v.s.	As for *X*	As for *X*
Spination of ventral sucker	+, 2 rows	+, no. ?	+, 2–3 rows
Intestinal caeca	Extend to posterior end of body	As for *X*	As for *X*
Excretory formula	2[(3)+(4+(2))]=18	As for *X*	As for *X*
Excretory commissure	.	.	.
Sporocyst birth-pore	+	+	?
Host	*Lymnaea stagnalis*	*L. megasoma*	*L. stagnalis*
Distribution and reference	Roath Park Lake, Cardiff, S. Wales (Rees, 1932)	Burt Lake, Michigan, U.S.A. (Miller, 1926)	Cheshire, England (Brown, 1931)

FURTHER DEVELOPMENT OF *CERCARIA X*

The penetration of cercaria into fish and its development in them has been confirmed and the host range of the metacercaria has been extended to include the Amphibia and the Mammalia. A list of the animals which were infected experimentally and of those in which a natural infection occurred is given in Table 3. The gradual sequence of changes in relation to time was studied by a series of dated experimental infections. The experimental animals used were *Leuciscus idus* and these were exposed to cercariae, allowed to recover and then killed and examined after a predetermined period. The infected fish were maintained in a large slate tank which had a continuous stream of tap water (approximate temperature 15° C.) passing through it. The fish were obtained from Surrey and ex-

Table 3. *Host distribution of* Diplostomulum X

Experimental	Natural
Phoxinus phoxinus	*Gasterosteus aculeatus*
Gasterosteus pungitius	*Cobitis taenia*
G. aculeatus	*Rutilus rutilus*
Salmo trutta	*Salmo trutta*
Leuciscus idus	*Anguilla vulgaris*
Lebistes reticulatus	
Rana temporaria (adults and tadpoles)	
Rattus norvegicus	

Morphologically mature and normal specimens were obtained from all hosts. The predominant secondary intermediate host in Roath Park Lake is *Gasterosteus aculeatus*. This species exhibited a natural infection rate of 80–90 % and the number of metacercariae in each lens varied from 1–100. The average number found in each lens was in the region 10–20.

amination previous to infection had shown them to be free of parasites. Although there are many descriptions of strigeid metacercariae, there have been very few detailed accounts of the development of the cercaria into the metacercarial form. The most detailed description is of the development of *Cercaria C* by Szidat (1924), which was later redescribed by Komiya in 1938.

7 *days after infection*

The cercariae present in the lens after this period had changed little in shape and size compared to the free-living forms. However, some of the organ systems characteristic of the cercarial stage had become modified. The penetration gland cells had disappeared, although the smaller anterior ends of the ducts present in the anterior organ still remained. The cytoplasm had become granular and contained a large number of globules and, because of this change in opacity, the gut was very indistinct with only the pharynx showing clearly. It was also impossible to observe the excretory system.

15 *days after infection*

The cytoplasm was more transparent at this stage and more details could be observed. The gut was well defined and consisted of oral sucker, pharynx, oesophagus and two intestinal caecae. The dilated ends of the penetration gland cells had disappeared completely at this stage (Text-fig. 4).

The excretory system had undergone differentiation into primary and secondary systems. The primary system was that of the cercaria with seven flame cells on either side. The excretory bladder had enlarged to form the secondary system, which consisted of two large tubular lateral extensions, a median collecting vessel and both anterior and posterior commissural vessels. The posterior collecting vessels were poorly developed.

20 *days after infection*

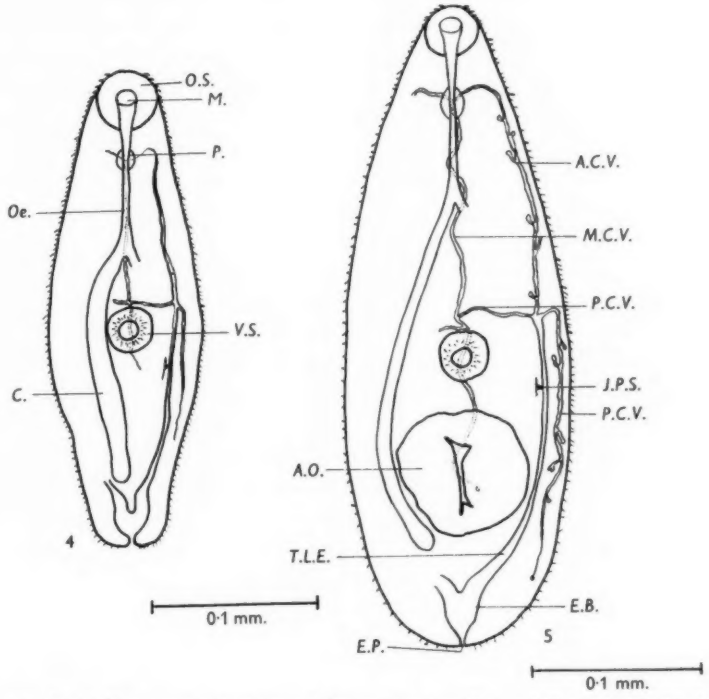
At this stage the proportion of cercarial to metacercarial characters had changed. An additional sucker, the posterior adhesive organ, had appeared between the ventral sucker and the posterior end of the body. Increase in body length had also occurred and the point of bifurcation of the oesophagus into intestinal caecae had changed from a position immediately in front of the ventral sucker to a position nearer the pharynx. The cuticle still exhibited the spination observed in the cercaria (Text-fig. 5).

The excretory system was more advanced than that of the previous stage. The posterior collecting vessels were better developed and, in common with the rest of the excretory system, possessed small evaginations with blind ends. At this stage neither flame cells nor calcareous bodies could be observed in these small diverticula. Only two pairs of flame cells, one at either end of the body, could be observed in the primary system.

46 days after infection

The chief difference between this stage and the previous one was the presence of a small hind-body projecting from the dorsal surface of the fore-body. This fore-body still possessed the cercarial spines.

The excretory system was similar to the previous stage, except for the presence of small calcareous bodies in the diverticula. In a specimen 44 days old, twenty-six flame cells were observed. The general outline of the fore-body had altered to



Text-fig. 4. Semi-diagrammatic view of the ventral surface of *Cercaria X* 15 days after localization in the lens of *Leuciscus idus*. Excretory system of the right side and left intestinal caecum have been omitted. Primary system not shown. Drawn from living material.

Text-fig. 5. *Cercaria X* 22 days after localization in the lens of *Leuciscus idus*. Other details as for Text-fig. 4.

a more oval shape and, on either side of the oral sucker, there was a groove corresponding to the future position of the lateral sucking organs. These, however, had not yet developed.

93 days after infection

After 93 days the larvae possessed the full complement of metacercarial characters and could be described as a typical diplostomulum. Spines were absent from the cuticle and the calcareous bodies were well developed.

It is apparent from these experiments that the change from cercaria to meta-

cercaria is a gradual one, involving the appearance of new structures and the loss of others, the full complement of metacercarial characters being attained after 50–60 days. It must be stressed that, in a given infection, the rates of development differed between individuals, so that the times given for the development of the various stages cannot be regarded as absolute. An additional factor is that the rate of development will vary in relation to the temperature of the environment at the time of the experiments.

It is very desirable to link the numerous descriptions of cercariae which exist to the other stages of the life-cycle and with this aim in mind it is essential to determine which cercarial characters are lost and which persist during development. Two typically cercarial characters which are lost during this period are the penetration gland cell system and the spines on the cuticle. The penetration gland cell system disappears soon after localization in the lens. The spines disappear more slowly and care must be taken in interpreting descriptions of immature larvae, as an account of the cercarial spination may be incorrectly included as a character of the metacercaria. The alimentary canal of the cercaria persists with a slight alteration in proportion, but the anterior penetration organ becomes modified to form the oral sucker of the metacercaria. The basic flame cell system persists, but an increase in the number of flame cells occurs. The excretory bladder enlarges to form the secondary excretory system of the metacercaria and, during the development of this system, it was observed that the calcareous bodies do not develop in association with flame cells, nor do they replace them.

During development there is an increase in size. The degree of the change is approximately indicated by Table 4, which contains data taken from a variety of sources. The figures for *Cercaria X* are of heat-killed material; those for 7 and 15 days after infection are of fresh, living material and those for the 45, 192 and 544 day figures were obtained from specimens fixed in mercuric-acetic fixative, stained in aceto-carmine and mounted in Canada balsam. This variation does not allow any detailed comparisons to be made, but a number of general conclusions are possible. Although extensive changes in size seem to occur during the early part of development, comparison of the last three columns of Table 4 (figures obtained from specimens treated in an identical manner) shows that little increase in size occurs after 45 days. During this period some changes occur in the various sucking organs. Although the ventral sucker seems to remain relatively constant the oral sucker and pharynx alter in proportion and the adhesive organ, which is a new structure, rapidly increases in size. These experiments indicate a gradual and continuous increase in size, and, because of this, the value of undated measurements of a larva isolated from the complete developmental sequence of that larval stage must be carefully assessed before being used as a basis for the separation of species. In addition, it is important that descriptions of new species should be accompanied, as far as possible, by the age of the particular stage, so that an accurate assessment can be made of the morphological features described. Thus in comparing this cercaria and metacercaria it is apparent that there do not appear to be any morphological characters which persist unaltered throughout development and which are sufficiently characteristic to link the two stages.

THE METACERCARIA—*DIPLOSTOMULUM X*

Cercaria X penetrates fish, undergoes an extensive migration, localizes in the lens of the eye and there develops into a diplostomulum. The only stages which have been recorded from the humours and other regions of the eye are the tailless cercariae undergoing their migration. There is also a fairly definite localization within the lens itself. In infected eyes the metacercariae were concentrated at the back of the lens adjacent to the retina and were distributed between the central hard core and the outer lens capsule (Pl. XIII, fig. 2).

Table 4. *Development of* Diplostomulum X

(Measurements in mm.)

Region		*Cercaria X*	Days after infection 7	15	45	192	544
Body	Length	0·188	0·196	0·217	0·241	0·266	0·28
		0·244	0·290	0·319	0·399	0·308	0·413
	Breadth	0·044	0·062	0·078	0·09	0·112	0·112
		0·055	0·081	0·118	0·135	0·147	0·182
Oral sucker	Length	0·040	—	—	0·032	0·023	0·031
		0·059	—	—	0·048	0·032	0·037
	Breadth	0·025	—	—	0·032	0·026	0·029
		0·034	—	—	0·035	0·030	0·034
Ventral sucker	Length	0·027	0·028	0·025	0·025	0·029	0·027
		0·034	0·040	0·031	0·029	0·031	0·034
	Breadth	0·029	0·028	0·021	0·025	0·029	0·029
		0·034	0·040	0·028	0·029	0·032	0·033
Adhesive organ	Length	—	—	0·031	0·035	0·046	0·02
		—	—	0·040	0·061	0·06	0·066
	Breadth	—	—	0·028	0·032	0·046	0·049
		—	—	0·037	0·058	0·058	0·069
Pharynx	Length	—	—	0·010	0·016	0·026	0·02
		—	—	0·015	0·025	0·031	0·029
	Breadth	—	—	0·010	0·009	0·011	0·011
		—	—	0·012	0·016	0·017	0·017

Maximum and minimum measurements are given. See text for further details.

At this stage the body of the parasite is composed of two well-defined regions; there is an anterior large fore-body and a posterior, relatively much smaller hind-body, which projects from the postero-dorsal surface of the fore-body. The fore-body is broad, thin and leaf-like in appearance in contrast to the hind-body, which, although it is capable of great expansion and contraction, is roughly conical in shape. Measurements of the various parts are given in Table 5.

The fore-body resembles that of other diplostomuli in possessing an oral sucker, lateral sucking organs, a ventral sucker and a posterior hold-fast or adhesive organ (Text-fig. 6). Both the lateral sucking organs and the adhesive organ are capable of extensive contraction and expansion. None of these suckers is armed with spines. The entire body is covered with a thin cuticle, which is devoid of spines and is approximately 0·007 mm. thick. The external surface of the cuticle is marked by a series of fine pits. Below the cuticle is a muscle layer composed of fine, outer

circular and stout, inner longitudinal fibres and within this is the parenchyma, which contains numerous large, pear-shaped cells with their ends prolonged into long, thin processes. These cells possess large, rounded nuclei and a refractile, granular cytoplasm and the distal ends of the cells run towards the cuticle and open there to the exterior, thus giving the pitted appearance to the cuticle mentioned earlier. Cells such as these seem to occur frequently in diplostomuli and have been mentioned by Chester Hughes (1929*a*), Hughes & Berkhout (1929) and Rees (1955). The possible function of these cells will be discussed later. The parasite possesses a well-differentiated alimentary canal composed of a short, prepharyngeal portion, a small muscular pharynx from which extends an oesophagus, which bifurcates in front of the ventral sucker into two long intestinal caeca. These caeca extend posteriorly on either side of the adhesive organ and in older metacercariae extend into the hind-body. The caeca were generally filled with small granules suspended in a fluid medium.

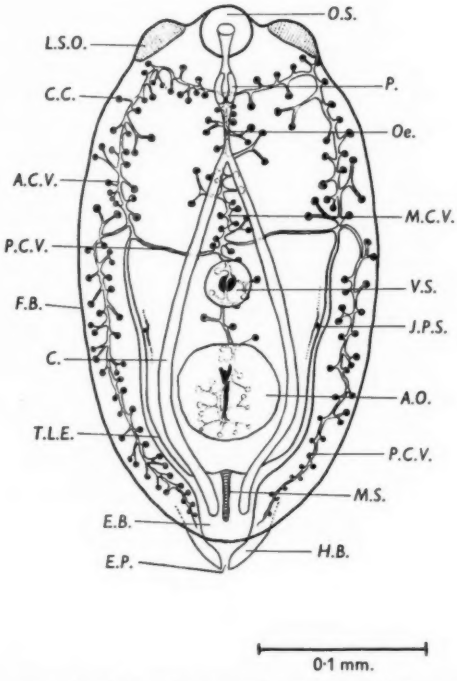
Table 5. *Measurements (mm.) of* Diplostomulum X, 544 *days after infection*

Experimental host: *Leuciscus idus* specimens fixed in hot mercuric acetic. Stained acetocarmine. Mounted in balsam

		Min.	Max.	Mean (10 specimens)
Body	Length	0·28	0·413	0·347
	Breadth	0·112	0·182	0·145
Oral sucker	Length	0·031	0·037	0·034
	Breadth	0·029	0·037	0·031
Ventral sucker	Length	0·024	0·034	0·028
	Breadth	0·029	0·033	0·03
Pharynx	Length	0·02	0·029	0·024
	Breadth	0·011	0·017	0·013
Distance between pharynx and ventral sucker		0·095	0·159	0·139
Adhesive organ	Length	0·049	0·066	0·053
	Breadth	0·049	0·069	0·056

The excretory system (Text-fig. 6) is complex in structure and can be conveniently divided into three regions: (*a*) the excretory bladder proper, (*b*) a system of wide trunks described as the reserve bladder or secondary system, and (*c*) a superficial system bearing flame cells described as the primary system by some authors and representing the excretory system of the cercaria. The greater portion of the triangular bladder is situated in the hind-body. A dorso-ventral septum divided the lumen of the bladder into two chambers (Pl. XIII, fig. 1), which communicate posteriorly and empty into a short duct, which opens to the exterior via a subterminal, dorsally situated, excretory aperture on the hind-body. The bladder is highly contractile and periodically fills and empties to the exterior. The basal corners of the bladder are prolonged anteriorly to give right and left tubular-lateral extensions, which run forward on either side of the adhesive organ to a position just in front of the ventral sucker. At this point each trunk divides into anterior and posterior collecting vessels. These posterior collecting vessels lie between the

tubular portion of the bladder and the body wall, diminishing in diameter until they disappear in the region of the bladder. The anterior collecting vessels run forward to a point opposite the base of the pharynx, where they turn inwards to the mid-line. The vessels from either side fuse just behind the pharynx and return posteriorly as a single, median collecting vessel terminating in a number of ramifications lying above the adhesive organ. At a level immediately anterior to the ventral sucker, the median vessel is linked on either side to the lateral portion of the excretory bladder by a fine duct—the posterior commissural vessel. All these vessels, except the posterior transverse commissural and the two lateral-tubular portions of the excretory bladder, give off short branches which dichotomize and



Text-fig. 6. Semi-diagrammatic view of the ventral surface of *Diplostomulum X* 544 days after the localization of *Cercaria X* in the lens of *Leuciscus idus*. The only part of the primary system included is the junction of the primary system with that of the secondary system. Outline drawn from fixed material.

ultimately end in small vesicles, each containing a calcareous body. The primary excretory system consists of a lateral vessel terminating blindly in the region of the pharynx and bladder on each side of the body. These lateral vessels are ultimately connected to flame cells, but the opacity of the cytoplasm and the presence of numerous calcareous bodies rendered the enumeration of flame cells impossible.

The earliest record of a diplostomulum in this country is that by Gulliver (1870,

1872) who gave a brief description of a metacercaria from the brain of *Petromyzon planeri*. This was probably related to *Diplostomulum* (*tetracotyle*) *petromyzontis* described by Brown (1899) from the brain of Ammocoetes (host species not given). Nicoll (1912) described some diplostomuli from the mesenteric fat of the three-striped snake (*Tropidonotus ordinatus sirtalis*). This, however, was not an indigenous species. In 1927 Ashworth & Bannerman gave a detailed account of *Diplostomulum phoxini* from the brain of the minnow. More recently Rushton (1937, 1938), Baylis (1939) and Dawes (1952) have recorded the occurrence of diplostomuli in fish and described the resultant blindness. There is throughout these papers an assumption that the larva described is *D. spathaceum*. However, Lal (1953) records a new species *D. trutti* from the lens of the trout. In America several species have been recorded and these will be referred to later. The first account of any experimental work in this country was that of Baylis & Taylor (1930) on *Cercaria X*. More recently, Rees (1955) showed by experimental infection that *Diplostomulum phoxini* was the metacercaria of *Diplostomum pelmatoides* (Dubois).

During the study of the literature it became apparent that precise identification of isolated larvae is very difficult. Structurally the species are very similar and differentiation has been based on measurements, shape, spination and host and tissue specificity. As observed in the section on development the value of the first two characters is greatly diminished unless the age of the larva is known. Biological characteristics such as specificity are equally difficult to interpret. In the case of *Cercaria X* it is possible to infect fish, Amphibia and mammals, but development into a metacercaria will take place only in the lens of the eye. This rigid tissue specificity is fairly characteristic of cercaria-metacercaria relationships of this type and generally species that localize in the eye do not develop in the brain and vice versa. Although hundreds of naturally infected fish were examined, and many experimental infections were performed, the metacercariae of *Cercaria X* were never recovered from any part of the body other than the eye. A number of metacercariae exhibiting similar tissue specificity are known and these are listed below:

Diplostomulum gigas	Eye, lens only. Hughes & Berkhout, 1929.
D. huronense	Eye, vitreous humours, 9–18 % in lens. Hughes & Hall, 1929.
D. scheuringi	Eye, humours only, never in lens. Hughes, 1929*b*.
D. spathaceum	Eye, various regions. Hughes, 1929.
D. trutti	Eye, mostly from vitreous humours. Lal, 1953.
D. phoxini	Brain, but small proportion occur in lens. Rees, 1955.
D. murrayensis	Eye, lens. Johnston & Simpson, 1939.

All these metacercariae occur in fish and are very similar in structure. Species distinction has been based on size and proportion of the various parts of the body and on the shape and clarity of the anterior lateral adhesive organs. In the American papers by Hughes, Berkhout and Hall distinction between the American species and *D. spatheceum* was based on Fraipont's (1880) observation that the lateral extensions of the excretory bladder possessed diverticula containing calcareous

bodies. A more recent study by Komiya (1938) has shown that in this respect Fraipont's observations were incorrect and that *D. spathaceum* was similar to all other species in the absence of calcareous bodies from the lateral extensions of the bladder. In some cases further details of the life cycle are known and these enable an accurate differentiation to be made. *D. gigas* is the metacercaria of *Cercaria flexicauda* (Cort & Brooks, 1928) and the adult, *Diplostomum flexicauda*, occurs in the intestine of *Larus argentatus* (Van Haitsma, 1931*b*). *Diplostomulum huronense* is the metacercaria of *Proalaria huronensis* (La Rue, 1927) and *Diplostomulum spathaceum* is the metacercaria of the well-known life cycle containing *Cercaria C*, with *Diplostomulum spathaceum* as the adult (Szidat, 1924). In the same genus, *Diplostomulum murrayensis* has been shown to be the metacercaria of *Cercaria murrayensis* with the adult *Diplostomum* located in the intestine of *Chlidonias leucopareia* (Johnston & Angel, 1941). Recently Rees (1955) has shown that *Diplostomulum phoxini* is the metacercaria of *Diplostomum pelmatoides*. Thus two species remain on which no further information is available. The original rather brief description of *D. scheuringi* was based on three live specimens and a collection of preserved material. Lateral suckers were not present on the specimens described and this suggests poor fixation or immature specimens. *D. trutti* is distinctive in possessing a papillated surface, whilst *D. huronense* and *D. gigas* are spined. From this comparison it seems fairly certain that *Diplostomulum X* has not been described previously under another name although it resembles *D. gigas* in size and shape. They differ morphologically in the absence of spines from the cuticle of *Diplostomulum X*, although a more certain differentiation is that the cercariae, although fairly similar in structure, possess different flame-cell formulae. *Cercaria X* possesses flame cells arranged as in the formula

$$2[(2+1)+(1+1+1+1+(2))]=18,$$

while in *C. flexicauda* the arrangement is

$$2[(3)+(3+(2))]=16.$$

HOST-PARASITE RELATIONSHIPS

Generally in any helminth infection the parasites have an adverse effect on the host. The precise effect will depend, amongst other things, upon the nature of the parasite and will even vary during the life cycle of that parasite. Thus, in this study of sporocyst, cercaria and metacercaria it has been possible to observe the different effects produced during parasitism by these stages.

(*a*) *Sporocyst*

As mentioned earlier, the secondary sporocysts localize in the connective tissue of the digestive gland. They multiply in this region and thus produce compression of the hepatic tubules. The state of tension which exists is easily demonstrated by pricking the tunica of an infected digestive gland. Immediately masses of wriggling sporocysts emerge. The histological changes include loss of the pseudo-cuticle covering the hepatic epithelium breakdown of the distal cell membrane and the

streaming of the cell contents into the lumen of the hepatic tubule. In some cases the epithelium was completely eroded down to the basement membrane. The destruction of the ciliated epithelium lining the small hepatic ducts was also observed. In the calcium cells the cytoplasm shrank from the cell membrane and the nucleus became pycnotic.

These observations are similar to those described by Faust (1917, 1920), Agersborg (1924), Dubois (1929), G. Rees (1931), W. J. Rees (1936) and Rothschild (1936). In contrast to my own observations and those of earlier workers, Pratt & Barton (1941) and Pratt & Lindquist (1943) describe parasitism of a gastropod by a larval trematode in which no destruction of the hepatic epithelium occurred. The only feature they recorded was a reduction in the number of tubules.

(*b*) *The cercariae*

The cercariae actively penetrate the second intermediate host and undergo extensive migrations through the tissues. Extension of the body drives the anterior spines into the tissues and the various rows of backwardly pointing spines allow the body to move in one direction only. In addition, the armed, ventral sucker forms an efficient anchorage to the tissue, so that sufficient thrust may be developed to force the anterior end into the tissues. In additon to this mechanical equipment the cercaria possesses four penetration gland cells and there is little doubt that migration is aided by the dissolution of the tissues by the secretions from these gland cells. The combined action of these agents on connective tissue and cartilage is illustrated by Pl. XIII, figs. 6, 7, which represent *Cercaria X* migrating through the subcutaneous tissues and through the optic capsule of *Gasterosteus aculeatus*.

Little is known about the nature of these secretions. Davis (1936) extracted and demonstrated the presence of a 'lytic' substance in the bodies of *Cercaria flexicauda*. Later, Levine, Garzoli, Kantz & Killough (1948) demonstrated the presence of a hyaluronidase in extracts of the cercaria of *Schistosoma mansoni* and suggested that the presence of this enzyme aids in the dissolution of the host tissues and would thus facilitate migration. Histochemical and biochemical studies on the cercaria of *S. mansoni* and *Schistosomatium douthitti* by Lewert & Lee (1954, 1956) demonstrated the presence of a collagenase activity in extracts of the cercariae. Similar tests with an unidentified strigeid cercaria were negative. These authors were also able to correlate marked changes in the glyco-protein containing material of the skin with the penetration and migration of the schistosome cercariae.

(*c*) *The metacercaria*

For the first few weeks within the lens continuous growth takes place. The metacercaria possesses a functional oral sucker and a well-developed alimentary canal, and active ingestion of the tissues by these larvae occurs. They localize in the outer region of the lens between the hard core and outer capsule.

One of the most characteristic features of the infected lens was that each parasite lay in a cavity, the outline of which was almost identical with the shape of the parasite itself. This can be seen in Pl. XIII, fig. 3. If one assumes that the destruction

of tissue is effected only by the activities of the oral sucker, it is difficult to explain how such cavities could have arisen by the haphazard ingestion of lens tissue. An alternative suggestion is that the parasite secreted, or excreted, a substance which reduced the normally firm tissue to a semi-fluid consistency, so that it readily conformed to the shape of the parasite body. The only observed structures which could be related to such a secretion were the gland cells described in the parenchyma. In some sections of lens which had been fixed in mercuric acetic and stained with Heidenhain's haematoxylin, the cuticle of the metacercaria was covered with globules of a substance which stained strongly with the haematoxylin. From various sections it was apparent that the globules, which varied in size, were formed by the coalescence of the secretions of the gland cells. Dissection of infected lenses revealed that the normally firm lens had become reduced to a granular, mushy consistency and microscopic examination showed that the lens fibres were quite free. Although no histochemical tests have been performed and the evidence is very circumstantial, it is tentatively suggested that the gland cells of the metacercaria produce a secretion whose activity may affect the lens tissue in some way producing the results described above.

The general result of these conditions is that the lens becomes cloudy and opaque and, although the eye may be still sensitive to changes of illumination, the formation of images is probably impossible. In addition to these changes, the lens was frequently elongated in the retinal-conjunctival plane and often it protruded through the iris. The exact effect on the host depended on the size of the lens and the number of parasites present. In two specimens of *Leuciscus idus*, which had been parasitized for 144 and 454 days, respectively, the lenses were markedly abnormal and, in addition to the features described above, a tumour-like growth was present on each lens. Although hundreds of naturally infected and many experimentally infected fish were examined, these conditions were only found in the two fish mentioned. This may be due to the fact that under normal conditions a fish so severely affected would not survive for long as it would be unable to avoid the attacks of predators.

HISTOCHEMICAL STUDIES

The presence and distribution in the sporocyst and cercarial stage of glycogen, lipase, acid phosphatase and alkaline phosphatase were investigated by using sections of heavily-infected digestive gland of *Lymnaea stagnalis*.

(*a*) *Enzymatic studies*

All attempts to demonstrate lipase activity at pH 7·4 and acid phosphatase activity at pH 5·0 were unsuccessful. It is possible that the concentration of both these enzymes was too low to survive the processing involved in the techniques. However, a well-defined alkaline phosphatase reaction was obtained in the cuticle of the sporocyst. Developing and mature cercariae were negative. As a result of studies on *Taenia pisiformis* and *Moniezia expansa* (Erasmus, 1957), it has been suggested that the presence of a cuticle exhibiting alkaline phosphatase activity is

connected with the passage of materials through the cuticle. It is especially significant that similar conditions, the absence of a gut and the associated passage of all metabolites through the cuticle, exist in the trematode sporocyst and it is possible that the enzymes present serve a similar function.

(*b*) *Glycogen*

Abundant glycogen was demonstrated in the cells of the sporocyst wall and in the developing cercariae. Within the fully developed cercariae there seemed to be several distinct regions of glycogen concentration. In the body, glycogen was mainly concentrated in the region immediately in front of the ventral sucker and also at the extreme posterior end. The greatest concentration occurred in the tail-stem. Each caudal body contained a mass of glycogen granules and, as there are twenty to thirty pairs of bodies in the tail-stem, the amount of glycogen stored here is quite considerable. The standard histochemical tests on fixed material were confirmed by staining newly emerged cercariae with iodine. With this test the bodies stained a reddish brown.

The life span of the free-swimming cercaria is quite short, approximately 24 hr. at 20° C. and during this period the caudal bodies degenerate into a mass of small granules. This is a characteristic change which has frequently been recorded, but no satisfactory explanation of it has been given. Immediately after emergence the caudal bodies, as indicated by the iodine tests, are rich in glycogen, but after 24 hr. the caudal bodies have disintegrated and a positive iodine reaction is no longer given (Pl. XIII, figs. 4, 5). Thus it seems probable that the caudal bodies represent large cells rich in glycogen reserves, which are utilized by the cercaria during its active free-swimming period.

COMPLETION OF THE LIFE CYCLE

Infection of the final host can only occur by the ingestion of parasitized fish. The search for the final host, which must necessarily be an animal that frequently feeds on fish, was conducted along two lines. Because of this dietary restriction it was thought that birds constituted the most likely section of the fauna liable to harbour the adult. Consequently, specimens of ducks, moorhens and seagulls which frequented the habitat were obtained and carefully examined for strigeid trematodes. None were found.

The second approach was that of carefully controlled laboratory infection experiments. Chicks, ducklings and rats were fed with heavily infected lenses and then killed after varying periods. Unfortunately none of these experiments were successful.

RELATIONSHIPS

Many pharyngeal distome longifurcous cercariae have been described and a number of attempts (Cort, 1917; Sewell, 1922; Miller, 1926; Cort & Brooks, 1928) at classification have been published. Sewell (1922) formed in his cercarial classification a division 'Group 2, Series 1', for such forms and Miller (1926) formed a

group 'pharyngeal, longifurcate, distome furcocercariae' for these organisms. Both workers went no further than this, mainly because of the lack of accurate descriptions. Miller listed thirty species in his group, but only twelve of these records included information concerning the excretory system and were sufficiently detailed for comparison with modern descriptions. Dubois (1929) increased the list of species with flame-cell data to twenty-nine, and Cort & Brooks (1928), working on the cercariae of Douglas Lake, Michigan, suggested that two groups 'Proalaria' and 'Strigea' could be formed, which would be based on the position of the penetration gland cells and the future development of the cercaria. The 'Proalaria' contained forms with the penetration gland cells posterior to the ventral sucker and which penetrated vertebrates to form a non-encysted diplostomulum larva. The 'Strigea' included cercariae with the penetration gland cells anterior to the ventral sucker and which penetrated invertebrates and developed into a tetracotyle larva which generally encysted.

Since the original differentiation into these groups, many cercariae and metacercariae have been described and a few life cycles have been completed. Some of the details of these cycles are given in Table 6. From a study of this table it is apparent that the two groups originally proposed by Cort & Brooks have little significance, but that the species in which there are only four penetration gland cells posterior to the ventral sucker, and in which there occurs a diplostomulum in the eye of fish form a compact group giving rise to the adult genus *Diplostomum* v. Nordmann, 1832. In this adult genus Dubois (1938, 1953) lists twenty species, of which nine have been recorded in Europe from the Lari (three species), Alcae (two species), Anseres (four species) and the Gauiiformes (one species). Because of the uniformity of this group, to which *Cercaria X* clearly belongs, it seems fairly probable that the adult genus of this cercaria is *Diplostomum*, but in the absence of experimental evidence it is impossible to suggest the particular species or the final host.

SUMMARY

1. *Cercaria X* (Baylis & Taylor, 1930) and its sporocyst have been redescribed with some additions to the original descriptions.

2. The details of the development of the cercaria into a metacercaria of the diplostomulum type have been obtained and the anatomy of the mature metacercaria is described in detail for the first time. It is proposed to name the metacercaria *Diplostomulum X*. Development of the cercaria into a metacercaria takes from 40 to 90 days and the metacercariae have been shown to survive for a period of 544 days after the time of infection. This development has been obtained in the lenses of rats, frogs and a wide variety of fish. Development is not completed in any other region of the host's body.

3. The effect of the sporocyst, migration of the cercariae, and the metacercaria on the appropriate host tissues has been considered. All these stages produce extensive changes in the host tissues.

4. Histochemical tests have shown the presence of alkaline phosphatase activity in the cuticle of the sporocyst. The glycogen storage regions of the cercaria are

Table 6. *The life cycles of some* members of the superfamily* Strigeides *Dubois* 1936

Adult	*Cercaria*	Penetration gland cells of Cercaria: Anterior to ventral sucker	Penetration gland cells of Cercaria: Posterior to ventral sucker	Ventral sucker	Flame-cell formula (*Cercaria*)	Mesocercaria	Metacercaria	Reference
Subfamily: Strigeinae Railliet, 1919								
Apharyngostrigea pipientis	*C. pipientis*	2	4	+	2[(2+2)+(2+2+(2))]=20	.	*Tetracotyle* (tadpole)	Olivier (1940)
A. ibis	*C. ibis*	2	4	+	2[6+(1)]=14	.	*Tetracotyle* (tadpole)	Azim (1935)
Subfamily: Cotylurini Dubois, 1936								
Cotylurus communis	*C. michiganensis*	.	6	+	2 [(2)+(4+(2))]=16	.	*Tetracotyle* (fish)	Van Haitsma, 1930
C. cornutus	*C. strigeae tardae*	4	.	+	2 [(4)+(3+(2))]=18	.	*Tetracotyle* (snail and leech)	Szidat, 1924; Mathias, 1922
C. flabelliformis	*C. flabelliformis*	4	.	+	2 [(4)+(4+(2))]=20	.	*Tetracotyle* (snail)	Van Haitsma, 1931*a*; Olivier & Cort, 1941
Apatemon gracilis	*C. gracilis*	.	8	+	2 [(1+1)+(2+2+(1))]=14	.	*Tetracotyle* (leech)	Szidat, 1929, 1931
A. intermedius	*C. leesoni*	2	6	+	2 [(2)+(2+2+(1))]=14	.	*Tetracotyle* (leech)	Johnston & Angel 1951; Johnston & Beckwith 1947
Subfamily: Diplostominae Monticelli, 1888								
Diplostomum spathaceum	*C. 'C'*	.	4	+	2 [(3)+(3) (2))]=16	.	*Diplostomulum* (fish, eye)	Szidat, 1924
D. flexicaudum	*C. flexicauda*	.	4	+	2 [(3)+(3+(2))]=16	.	*Diplostomulum* (fish, eye)	Van Haitsma, 1931*b*
D. murrayensis	*C. murrayensis*	.	4	+	2 [(3)+(3+(2))]=16	.	*Diplostomulum* (fish, eye)	Johnston & Angel 1941; Johnston & Cleland, 1938
D. pelmatoides	*C. pelmatoides*	.	4	+	2 [(3)+(3+(2))]=16	.	*Diplostomulum* (fish, brain)	Rees, 1955, 1956

Hysteromorpha triloba	*C. micradena*	2	2	+	2 [(4) + (4 + (2))] = 20	.	*Diplostomulum* (fish, muscle)	Olivier, 1940; Hugghins, 1953
Tylodelphys excavata	*C. excavata*	4	.	+	2 [(1 + 1) + (3 + (2))] = 14	.	*Tylodelphys* (frog)	Szidat, 1935
Posthodiplostomum minimum	*C. minimum*	2	4	R	2 [(1 + 1 + 2) + (2 + 2 + (2))] = 20	.	*Posthodiplostomulum* (fish)	Ferguson, 1936, 1940; Miller, 1923; Hunter, 1936
Uvulifer ambloplitis	*C. bessiae*	2	4	+	2 [(3) + (3 + (2))] = 16	.	*Neascus* (fish)	Hunter & Hunter 1934
Crassiphiala bulboglossa	*C. bulboglossa*	2†	4†	.	2 [(7 + (2))] = 18	.	*Neascus* (fish)	Hoffman, 1956
Subfamily: Alariinae Hall et Wigdor, 1918								
Fibricola texensis	*C. texensis*	4	.	+	2 [(3) + (2 + (1))] = 12	.	*Diplostomulum* (Tadpole)	Chandler, 1942
Pharyngostomum cordatum	*C. cordatum*	4	.	+	2 [(2) + (2 + (1))] = 10	.	*Diplostomulum* (Tadpole)	Wallace, 1939
Alaria canis	*C. canis*	4	.	+	2 [(2 + 2 + 2) + (2 + 2 + (2))] = 24	+ frog	*Diplostomulum* (lung-mammal)	Pearson, 1956
A. arisaemoides	*C. arisaemoides*	4	.	+	2 [(2 + 2 + 2) + (2 + 2 + (2))] = 24	+ frog	*Diplostomulum* (lung-mammal)	Pearson, 1956
A. marcianae	*C. marcianae*	4	.	.	2 [(2 + 2 + 2) + (2 + 2 + (2))] = 24	+ frog	*Diplostomulum* (mouse–kitten)	Cuckler, 1949
A. mustelae	*C. mustelae*	2	2	+	2 [(3) + (2 + (2))] = 14	+ frog	*Diplostomulum* (mice and rats)	Bosma, 1934
A. intermedia	*C. intermedia*	2	2	.	2 [(2 + 2 + 2) + (2 + 2 + (2))] = 24	+ frog	*Diplostomulum* (rats and mice)	Odlaug, 1940

* Only those species have been described in which there was complete information on all stages.
† Position cannot be clearly defined in absence of ventral sucker.
R, Rudimentary.

described and it has been demonstrated that the caudal bodies in the tail-stem contain large amounts of glycogen which are utilized during the free-swimming period.

5. Attempts at the completion of the life cycle were unsuccessful.

Grateful acknowledgement is expressed to Mr Nelmes, Superintendent of Parks, Cardiff, who provided generous collecting facilities; to the Agricultural Research Council, as part of this work was carried out during the tenure of a research studentship; to Professor J. Brough for his interest and advice; to Dr E. M. Sheppard for providing me with a large proportion of the literature on strigeid metacercariae from her own private collection of papers; to Dr G. Rees for allowing me to use unpublished information concerning the life cycle of *Diplostomum pelmatoides*; and to Dr J. Griffith for valuable assistance in the field.

KEY TO LETTERING OF FIGURES

A.C.V.	Anterior collecting vessel	*L.S.O.*	Lateral sucking organ
Ant.O.	Anterior organ	*M.*	Mouth
A.O.	Adhesive organ	*M.C.V.*	Median collecting vessel
A.P.G.C.	Aperture of penetration gland cell	*M.S.*	Median septum
C.	Caecum	*O.C.*	Oral cap
C.B.	Caudal body	*Oe.*	Oesophagus
C.C.	Calcareous corpuscle	*O.S.*	Oral sucker
E.B.	Excretory bladder	*P.*	Pharynx
E.P.	Excretory pore	*P.C.V.*	Posterior collecting vessel
F.	Furca	*P.G.C.*	Penetration gland cell
F.B.	Fore-body	*T.L.E.*	Tabular lateral extension
H.B.	Hind-body	*T.S.*	Tail-stem
J.P.S.	Junction of primary and secondary systems	*V.S.*	Ventral sucker

REFERENCES

AGERSBORG, H. P. K. (1924). Studies on the effect of parasites upon the tissues. I. With special reference to certain gastropod molluscs. *Quart. J. Micr. Sci.* **68**, 361–403.

ASHWORTH, J. H. & BANNERMAN, J. C. W. (1927). On a Tetracotyle (*T. phoxini*) in the brain of the minnow. *Trans. Roy. Soc. Edinb.* **55**, 159–74.

AZIM, M. A. (1935). Entwicklungsgeschichte von *Apharyngostrigea ibis* n.sp. und seine Entwicklung aus *Cercaria Apharyngostrigea ibis* n.sp. in dem Reiher *Ardeola ibis*. *Z. Parasitenk.* **7**, 608–14.

BAYLIS, H. A. (1939). A larval trematode (*Diplostomulum volvens*) in the lens of the eye of a rainbow trout. *Proc. Linn. Soc. Lond.* **151**, 130.

BAYLIS, H. A. & TAYLOR, E. L. (1930). Observations and experiments on a dermatitis producing cercaria and other cercaria from *Lymnaea stagnalis* in Great Britain. *Trans. R. Soc. Trop. Med. Hyg.* **24**, 219–44.

BOLLES LEE (1946). *The Microtomist's Vade-mecum*, 10th ed. London: J. and A. Churchill Ltd.

BOSMA, N. (1934). The life-history of the trematode *Alaria mustelae*. *Trans. Amer. Micr. Soc.* **53**, 116–53.

BROWN, A. W. (1899). On *Tetracotyle petromyzontis*, a parasite of the brain of *Ammocoetes*. *Quart. J. Micr. Sci.* **41**, 489–99.

BROWN, F. J. (1926). Some fresh-water larval trematodes, with contribution to their life-history. *Parasitology*, **18**, 21–34.

BROWN, F. J. (1931). Some fresh-water larval trematodes from Cheshire. *Parasitology*, **23**, 88–99.

CARLETON, H. M. & LEACH, E. H. (1938). *Histological Technique*, 2nd ed. London: Oxford University Press.

CHANDLER, A. (1942). The morphology and life-cycle of a new strigeid trematode *Fibricola texensis*. *Trans. Amer. Micr. Soc.* **61**, 156–67.

CORT, W. W. (1917). Homologies of the excretory system of the fork-tailed cercariae. *J. Parasit.* **4**, 49–57.

CORT, W. W. & BROOKS, S. T. (1928). Studies on the trematode family Strigeid, No. VIII. Studies on the holostome cercariae from Douglas Lake, Michigan. *Trans. Amer. Micr. Soc.* **47**, 179–221.

CUCKLER, A. C. (1949). Morphological and biological studies on certain strigeid trematodes of mammals. *Summaries of Ph.D. Theses*. University of Minnesota, Minneapolis, Minn. **4**, 45–7.

DAVIS, D. J. (1936). Report on the preparation of a histolytic ferment present in the bodies of cercariae. *J. Parasit.* **22**, 108–10.

DAWES, B. (1952). Trematode life-cycle enacted in a London pond. *Nature, Lond.*, **170**, 72–3.

DUBOIS, G. (1929). Les cercaires de la région de Neuchâtel. *Bull. Soc. neuchâtel. Sci. nat.* **53**, 1–177.

DUBOIS, G. (1938). Monographie des Strigeida (Trematoda). *Mém. Soc. neuchâtel. Sci. nat.* **6**, 1–535.

DUBOIS, G. (1953). Systématique des Strigeida. Complément de la *Mém. Soc. neuchâtel. Sci. nat.* **8**, 1–141.

ERASMUS, D. A. (1957). Studies on the phosphatase systems of Cestodes. Part 1. The enzymes present in *Taenia pisiformis* (Cysticercus and adult). *Parasitology*, **47**, 70–80.

ERASMUS, D. A. (1957). The phosphatase systems of Cestodes. Part 2. Studies on *Cysticercus tenuicollis* and *Moniezia expansa* (adult). *Parasitology*, **47**, 81–91.

FAUST, E. C. (1917). Notes on the cercariae of the Bitter Root Valley, Montana. *J. Parasit.* **3**, 105–23.

FAUST, E. C. (1920). Pathological changes in the gastropod liver produced by fluke infections. *Johns Hopk. Hosp. Bull.* **31**, 79–84.

FERGUSON, M. S. (1936). Experimental studies on *Neascus vancleavei*. *J. Parasit.* **22**, 544.

FERGUSON, M. S. (1940). Excystment and sterilization of metacercariae of the avian strigeid trematode *Posthodiplostomum minimum*, and their development into adult worms in sterile cultures. *J. Parasit.* **26**, 359–72.

FRAIPONT, J. (1880). Recherches sur l'appareil excréteur des trématodes et des cestodes. *Arch. Biol., Paris*, **1**, 415–56.

GLICK, D. (1949). *Techniques of Histo- and Cytochemistry*, 1st ed., 531 pp., 159 text-figs. New York: Interscience Publishers.

GULLIVER, G. (1870). On certain points in the anatomy and economy of the lampreys. *Proc. Zool. Soc. Lond.* **10**, 844–50.

GULLIVER, G. (1872). Note without title. *Quart. J. Micr. Sci.* **12**, 102–3.

HARPER, F. W. (1931). On the structure and life-histories of British fresh-water furcocercariae. *Parasitology*, **23**, 310–25.

HESSE, A. J. (1932). A description of two cercariae found in *Lymnaea pereger* in Scotland. *J. Helminth.* **1**, 227–36.

HOFFMAN, G. L. (1956). The life-cycle of *Crassiphiala bulboglossa* (Trematoda: Strigeida). Development of the metacercariae and cyst, and effect on the fish hosts. *J. Parasit.* **42**, 435–44.

HUGGHINS, E. J. (1953). Life history of a strigeid trematode. *Hysteromorpha triloba* (Rud. 1819), Lutz, 1931. *J. Parasit.* **39**, 15–16.

HUGHES, R. C. (1929*a*). Studies on the trematode family Strigeidae (Holostomidae). No. XIV. Two new species of Diplostomula. *Occ. Pap. Mus. Zool. Univ. Mich.*, no. 202. 1–29.

HUGHES, R. C. (1929*b*). Studies on the trematode family Strigeidae (Holostomidae), No. XIX. *Diplostomulum scheuringi* sp.nov. and *D. vegrandis* (La Rue). *J. Parasit.* **15**, 267–71.

HUGHES, R. C. & BERKHOUT, P. G. (1929). Studies on the trematode family Strigeidae (Holostomidae). No. XV. *Diplostomulum gigas*, sp.nov. *Pap. Mich. Acad. Sci.* **10**, 438–88.

HUGHES, R. C. & HALL, L. J. (1929). Studies on the trematode family Strigeidae (Holostomidae). No. XVI. *Diplostomulum huronense* (La Rue). *Pap. Mich. Acad. Sci.* **10**, 489–94.

HUNTER, G. W. (1936). Penetration of the common sunfish by the holostome cercaria *C. multicellulata*. *J. Parasit.* **22**, 542.

HUNTER, G. W. & HUNTER, W. S. (1934). The life history of the black grub of Bass, *Crassiphiala amblophitis* (Hughes). *J. Parasit.* **20**, 328.

JOHNSTON, T. H. & ANGEL, L. M. (1941). Life-cycle of the Trematode *Diplostomum murrayense* J. & C. *Trans. Roy. Soc. S. Aust.* **65**, 140–4.

JOHNSTON, T. H. & ANGEL, L. M. (1951). The morphology and life-cycle of the Trematode *Apatemon intermedius*, from the black swan. *Trans. Roy. Soc. S. Aust.* **74**, 66–78.

JOHNSTON, T. H. & BECKWITH, A. C. (1947). Larval trematodes from Australian freshwater molluscs. Part XI. *Rec. S. Austr. Mus.* **8**, 563–83.

JOHNSTON, T. H. & CLELAND, E. R. (1938). Larval trematodes from Australian terrestrial and freshwater molluscs. Part IV. *Cercaria* (Furcocercaria) *murrayensis* n.sp. *Trans. Roy. Soc. S. Aust.* **62**, 127–31.

JOHNSTON, T. H. & SIMPSON, E. R. (1939). The diplostomulum stage of *Cercaria murrayensis*. *Trans. Roy. Soc. S. Aust.* **63**, 230–7.

KOMIYA, Y. (1938). Die Entwicklung des Excretionsystems einiger Trematodenlarven aus Alter und Elbe, nebst Bemerkungen über ihren Entwicklungszyklus. *Zeit. Parasitenk.* **10**, 340–85.

LAL, M. B. (1953). A new trematode metacercaria from the eyes of Trout. *Nature, Lond.*, **171**, 130.

LEVINE, M. D., GARZOLI, R. F., KUNTZ, R. E. & KILLOUGH, J. H. (1948). On the demonstration of hyaluronidase in cercariae of *Schistosoma mansoni*. *J. Parasit.* **34**, 158–62.

LEWERT, R. M. & LEE, C. L. (1954). Studies on the passage of helminth larvae through host tissues. *J. Infect. Dis.* **95**, 13–51.

LEWERT, R. M. & LEE, C. L. (1956). Quantitative studies of the collagenase-like enzymes of cercariae of *Schistosoma mansoni* and the larvae of *Strongyloides ratti*. *J. Infect. Dis.* **99**. 1–14.

MATHIAS, P. (1922). Cycle évolutif d'un trématode holostomidae (*Strigea tarda*, Steenstrup). *C.R. Acad. Sci., Paris*, **175**, 599–602.

MILLER, H. M. (1923). Notes on some furcocercous larval trematodes. *J. Parasit.* **10**, 35–46.

MILLER, H. M. (1926). Comparative studies on furcocercous cercariae. *Illinois Biol. Monogr.* **10**, 1–112.

NICOLL, W. (1912). On two new larval trematodes from the striped snake (*Tropidonotus ordinatus sirtalis*). *Proc. Zool. Soc. Lond.* pp. 767–70.

ODLAUG, T. (1940). Morphology and life-history of the trematode *Alaria intermedia*. *Trans. Amer. Micr. Soc.* **59**, 490–510.

OLIVIER, L. (1940). Life-history studies on two strigeid trematodes of the Douglas Lake region, Michigan. *J. Parasit.* **26**, 447–77.

OLIVIER, L. & CORT, W. W. (1941). *Cercaria douglasi* (Cort, 1917) and its relationship to the cercaria of *Cotylurus flabelliformis* (Faust, 1917). *J. Parasit.* **27**, 343–6.

PEARSON, J. C. (1956). Studies on the life-cycles and morphology of the larval stages of *Alaria arisaemoides* Augustine and Uribe, 1927 and *Alaria canis* La Rue and Fallis, 1936 (Trematoda: Diplostomidae). *Canad. J. Zool.* **34**, 295–387.

PRATT, I. & BARTON, G. D. (1941). The effects of four species of larval trematodes upon the liver and ovotestis of the snail, *Stagnicola emarginata angulata* (Sowerby). *J. Parasit.* **27**, 283–8.

PRATT, I. & LINDQUIST, W. D. (1943). The modification of the digestive gland tubules in the snail *Stagnicola* following parasitization. *J. Parasit.* **29**, 176–81.

REES, G. (1931). Some observations and experiments on the biology of larval trematodes. *Parasitology*, **23**, 428–41.

REES, G. (1932). An investigation into the occurrence, structure and life-histories of the trematode parasites of four species of *Lymnaea* and *Hydrobia jenkinsi* (Smith) in Glamorgan and Monmouth. *Proc. Zool. Soc. Lond.* pp. 1–32.

REES, G. (1955). The adult and diplostomulum stage *Diplostomulum phoxini* (Faust) of *Diplostomulum pelmatoides* Dubois and an experimental demonstration of part of the life-cycle. *Parasitology*, **45**, 295–312.

REES, G. (1956). Personal communication.

REES, W. J. (1936). The effects of parasitism by larval trematodes on the tissues of *Littorina littorea* (Linne). *Proc. Zool. Soc. Lond.* pp. 357–72.

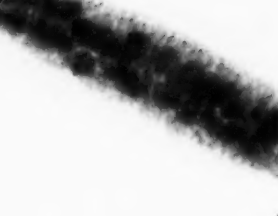
1

2

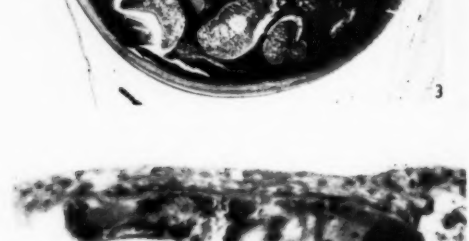
4

3

6



5



7

(*Facing p.* 335)

ROTHSCHILD, M. (1936). Gigantism and variation in *Peringia ulvae* Pennant 1777, caused by infection with larval trematodes. *J. Mar. Biol. Ass. U.K.* **20**, 537–46.

LA RUE, G. (1927). Studies on the trematode family Strigeidae (Holostomidae). No. V. *Proalaria huronensis* sp.nov. *Trans. Amer. Micr. Soc.* **46**, 26–35.

RUSHTON, W. (1937). Blindness in fresh-water fish. *Nature, Lond.*, **140**, 1114.

RUSHTON, W. (1938). Blindness in fresh-water fish. *Nature, Lond.*, **141**, 289.

SEWELL, R. B. S. (1922). Cercariae Indicae. *Indian. J. Med. Res.* **10**, 1–370.

SZIDAT, L. (1924). Beiträge zur Entwickelungsgeschichte der Holostomiden II. *Zool. Anz.* **61**, 249–66.

SZIDAT, L. (1929). Beiträge zur Entwicklungsgeschichte der Holostomiden III. Ueber zwei Tetracotyle aus Hirudineen und ihre Weiterentwicklung in Enten zu *Cotylurus cornutus* Rud. und *Apatemon gracilis* Rud. *Zool. Anz.* **86**, 133–49.

SZIDAT, L. (1931). Beiträge zur Entwicklungsgeschichte der Holostomiden IV. Die Cercarie des Entenparasiten *Apatemon* (*Strigea*) *gracilis* Rud und ihre Entwicklung im Blutgefassystem des Zwischenwirtes (*Herpobdella atomaria* Car.). *Z. Parasitenk.* **3**, 160–72.

SZIDAT, L. (1935). Warum wirft der Storch seine Jungen aus dem Nest? Ein Beitrag zur Brutbiologie und Oekologie des weissen Storches (*Ciconia ciconia* L.), sowie zur Kenntnis seiner Parasitenfauna. *J. Orn., Lpz.* **83**, 76–87.

VAN HAITSMA, J. P. (1930). Studies on the Trematode family Strigeidae (Holostomidae). No. XXI. Life-cycle and description of the cercaria of *Cotylurus michiganensis* (La Rue). *J. Parasit.* **16**, 224–30.

VAN HAITSMA, J. P. (1931*a*). Studies on the trematode family Strigeidae (Holostomidae), No. XXII. *Cotylurus flabelliformis* (Faust) and its life-history. *Pap. Mich. Acad. Sci.* **13**, 447–82.

VAN HAITSMA, J. P. (1931*b*). Studies on the trematode family Strigeidae (Holostomidae). No. XXIII. *Diplostomum flexicaudum* (Cort and Brooks) and stages in its life-history. *Pap. Mich. Acad. Sci.* **13**, 483–516.

WALLACE, F. G. (1939). The life-history of *Pharyngostomum cordatum* (Alariidae). *Trans. Amer. Micr. Soc.* **58**, 49–61.

EXPLANATION OF PLATE XIII

Fig. 1. T.S. hind body of *Diplostomulum X*. Note the median septum dividing the excretory bladder into two compartments. × 560.

Fig. 2. Sagittal section of the eye of *Gasterosteus aculeatus* infected with *Diplostomulum X*. Note the localization of the metacercariae to the region of the lens nearest the retina. Heidenhain's haematoxylin and Orange G. × 44.

Fig. 3. Tangential section of the eye of *Gasterosteus aculeatus* infected with *Diplostomulum X*. Note the cavities in which the parasites lie. Heidenhain's haematoxylin and Orange G. × 54.

Fig. 4. Caudal bodies in tail-stem of newly emerged specimens of *Cercaria X* stained with iodine. The caudal bodies stained a reddish brown and the rest of the tail was yellow. × 450.

Fig. 5. Tail-stem of *Cercaria X* 24 hr. after emergence from snail. There was no glycogen reaction; the entire tail stained yellow. × 550.

Fig. 6. L.S. of *Cercaria X* migrating through the subcutaneous tissues of *Gasterosteus aculeatus*. Note the cavity which has been produced by the parasite. Erhlich's haematoxylin and Eosin. × 380.

Fig. 7. Section showing *Cercaria X* penetrating the optic capsule of *Gasterosteus aculeatus*. Note the destruction of the capsule by the parasite. × 370.

(*MS. received for publication* 17. VI. 1957.—Ed.)

STUDIES ON *CREPIDOSTOMUM METOECUS* (BRAUN) AND *C. FARIONIS* (MÜLLER), PARASITIC IN *SALMO TRUTTA* L. AND *S. SALAR* L. IN BRITAIN

By J. D. THOMAS, B.Sc., Ph.D.

Zoology Department, University College of Ghana, Accra, Ghana

(With 10 Figures in the Text)

CONTENTS

		PAGE
I.	Introduction	336
II.	Materials and methods	336
III.	Observations	338
	Double infestation of host fishes with *C. metoecus* and *C. farionis*	338
	Taxonomy	341
	(*a*) Taxonomy of *C. metoecus*	341
	(*b*) Comparison between *C. metoecus* and *C. farionis*	344
	Geographical distribution of *C. metoecus* and *C. farionis*	344
	Degree of infestation and pathogenicity	345
	Host specificity and immunity	346
	Seasonal variation in numbers of parasites	346
	Life cycle	347
IV.	Discussion	347
V.	Summary	350
	References	351
	Key to abbreviations in figures	352

I. INTRODUCTION

During the period extending from November 1950 to February 1952, investigations were undertaken into the food, growth and helminth parasites of brown trout, *Salmo trutta* L., from four contrasted habitats in mid-Wales. The food and parasites of the salmon parr, *Salmo salar* L., and the eel, *Anguilla anguilla* (L.), were also studied. The investigation of the helminth parasites, from the ecological standpoint, received special attention in view of the paucity of knowledge concerning this subject. Most of the investigations into the parasites of fishes, particularly the earlier ones, have been contributions relating to taxonomy, geographical distribution and host specificity. The few papers that refer to the inter-relationships between fish parasites and their hosts include those by Pearse (1924), Van Cleave & Müeller (1934), Bangham (1944) and Frankland (1955).

II. MATERIALS AND METHODS

The four fish-sampling stations selected for these studies are situated in West Wales on three river systems (Fig. 1), stations A and B being on the Teify, C on the Rheidol and D on the Pysgotwr, a tributary of the Towy. The Teify and Rheidol flow into Cardigan Bay and the Towy into Carmarthen Bay.

The number of fishes examined, the percentage found infested with *Crepidostomum* spp. and the period over which the investigation extended is given in Table 1. From seven to sixty fishes from individual stations were examined at weekly intervals in the laboratory throughout the specified periods.

Each fish was given, prior to examination, a reference number, and the parasitological data concerning it were completed in accordance with the plan shown in

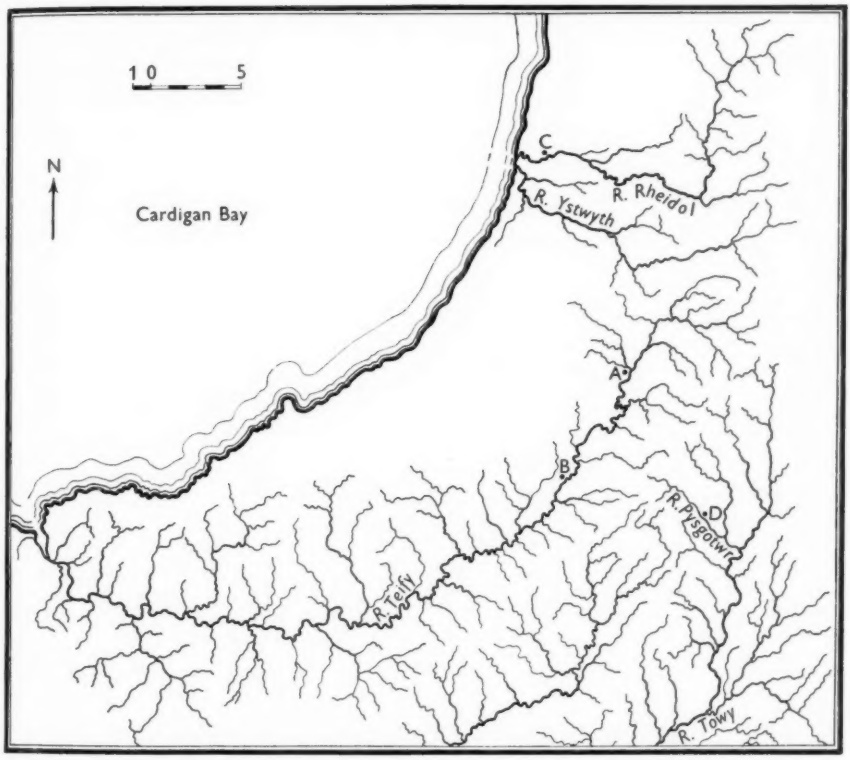


Fig. 1. Map of the rivers investigated, showing the position of the fish-sampling stations.

Table 1. *Number of fishes examined and the percentage found to be infested with* Crepidostomum *spp. at stations A, B, C and D*

Habitat	No. of fish examined. Percentage infested with *Crepidostomum* spp. in brackets: Trout	Salmon parr	Eels	Period of investigation
Teify, station A	215 (60·2)	—	88 (0)	Mar.–June, Sept.–Nov. 1951
Teify, station B	687 (81·0)	274 (33·9)	155 (0·65)	Jan.–Dec. 1951
Rheidol, station C	132 (79·0)	—	—	Nov.–Dec. 1951
Pysgotwr, station D	278 (70·5)	—	—	June–Aug. 1951, Jan.–Feb. 1952

Table 2. All the parasites were counted and grouped provisionally into monogenetic Trematodes, digenetic Trematodes, Cestodes, Acanthocephala, and Nematoda. The only digenetic trematodes encountered in the intestine were the two species of *Crepidostomum*. Unfortunately it was not realized at first that there were two species involved and consequently they were not counted separately. The ages of the trout were ascertained by examination of their scales and in this way it became possible to assemble the data shown in Tables 3 and 4 and Fig. 2. The data concerning the salmon parr are also given in Table 3, but no attempt was made to determine the age groups of these fishes.

Table 2. *Data relating to the parasites*

				No. of each class of parasites found in organs of host					
Ref. no.	Date	Host. sp.	Age of fish	Skin	Gills	Cardiac stomach	Pyloric stomach	Intestine	Urinary bladder

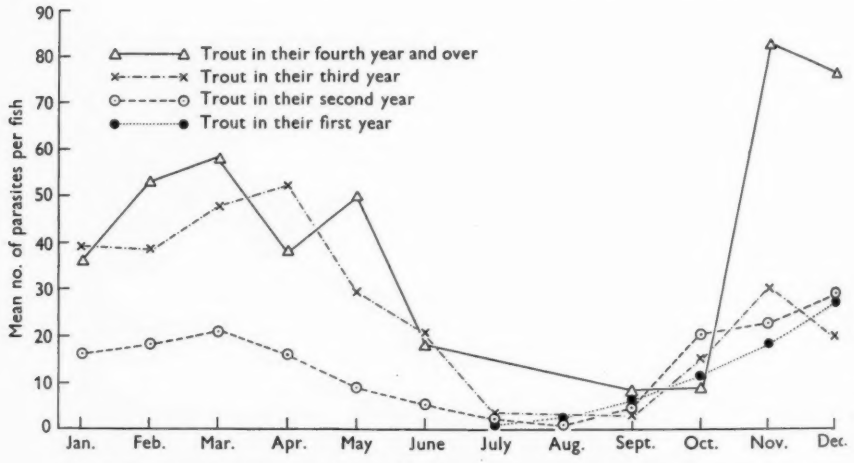


Fig. 2. The average number of *Crepidostomum* spp. trout of different ages from station B, on a monthly basis.

A large and representative proportion of the *Crepidostomum* species obtained from all four stations were fixed in Gilson's fluid under slight pressure from a coverglass. *In toto* preparations were stained in Coelestine Blue B and Mayer's paracarmine, and the transverse and sagittal serial sections in Ehrlich's haemotoxylin and eosin.

III. OBSERVATIONS

Double infestation of host fishes with Crepidostomum metoecus *and* C. farionis

During the present investigation the two species of *Crepidostomum* were found to occur together in the salmonid fishes examined. There was, however, some evidence of a habitat isolation in individual fish as *C. metoecus* occurred predominantly within

Table 3. *The occurrence of* Crepidostomum *spp. in trout of different ages and salmon parr from station B on the Teify*

(% F. indicates the percentage number of fish infested; Av., the average number of parasites per fish.)

Months	Trout										Salmon parr		
	1-year-old fish			2-year-old fish			3-year-old fish			Fish of 4 years and over			All ages		
	No. of fish	% F.	Av.	No. of fish	% F.	Av.	No. of fish	% F.	Av.	No. of fish	% F.	Av.	No. of fish	% F.	Av.
Jan.	—	—	—	28	100·0	15·9	31	100·0	39·5	23	100·0	36·0	8	75·0	6·0
Feb.	—	—	—	21	100·0	18·2	37	94·6	38·6	15	100·0	53·0	4	50·0	2·5
Mar.	—	—	—	15	100·0	20·7	12	100·0	48·2	15	100·0	58·3	8	87·5	8·3
Apr.	—	—	—	22	100·0	15·6	10	80·0	52·6	8	100·0	37·3	13	83·3	6·5
May	4	25·0	1·0	33	84·8	9·1	11	81·8	29·2	12	92·5	49·4	2	50·0	2·0
June	1	—	—	7	71·5	4·6	9	88·9	20·6	3	66·7	18·0	4	25·0	2·0
July	2	50·0	1·0	10	50·0	1·8	5	40·0	2·5	2	—	—	33	21·3	2·3
Aug.	26	11·7	2·3	8	12·5	1·0	2	—	—	—	—	—	25	12·0	2·0
Sept.	46	17·3	5·9	17	47·0	4·5	5	60·0	3·0	9	11·1	8·0	56	18·0	1·8
Oct.	29	62·7	11·6	21	76·2	20·4	14	56·8	14·9	5	60·0	9·3	66	21·2	3·3
Nov.	48	94·5	17·9	27	96·3	12·7	11	100·0	30·1	3	100·0	83·0	24	25·5	6·5
Dec.	38	100·0	26·6	21	100·0	28·7	13	100·0	19·9	3	100·0	17·0	31	35·5	3·8

Table 4. *The occurrence of* Crepidostomum *spp. in trout of different ages from stations A, C and D*

(% F. indicates the percentage number of trout infested; Av., the average number of parasites per fish.)

Months	1-year-old fish			2-year-old fish			3-year-old fish			Fish of 4 years and over		
	No. of fish	% F.	Av.	No. of fish	% F.	Av.	No. of fish	% F.	Av.	No. of fish	% F.	Av.
Station A												
Mar., Apr.	—	—	—	7	85·7	(16·0)	9	100·0	(52·5)	10	100·0	(70·5)
May	—	—	—	7	85·7	(9·7)	4	75·0	(37·5)	5	80·0	(28·8)
June	—	—	—	3	33·3	(31·0)	3	100·0	(28·7)	2	100·0	(36·5)
Sept.	9	0	0	1	0	0	1	0	0	—	—	—
Oct.	37	54·0	(13·5)	32	40·7	(6·6)	22	68·2	(19·4)	13	60·8	(16·5)
Nov.	21	66·5	(11·7)	10	100·0	(25·3)	7	85·7	(20·3)	4	100·0	(19·5)
Station C												
Nov.	45	60·0	10·8	12	83·3	(18·0)	1	100·0	(6·0)	—	—	—
Dec.	40	100·0	17·9	18	88·9	(24·6)	6	83·5	(30·2)	—	—	—
Station D												
June	2	0	0	23	100·0	(19·3)	3	100·0	(103·0)	—	—	—
July	1	100·0	(7·0)	91	68·1	(12·0)	5	100·0	(40·1)	2	0	0
Aug.	—	—	—	17	4·7	(4·0)	3	33·3	(9·1)	—	—	—
Jan.	—	—	—	14	100·0	(14·5)	32	100·0	(49·8)	4	100·0	(44·4)
Feb.	—	—	—	20	100·0	(15·6)	51	100·0	(40·2)	10	100·0	(77·4)

the pyloric caeca, the chief centre of digestion, while *C. farionis* generally occupied a more posterior station in the intestine.

Taxonomy

As only brief descriptions of *C. metoecus*, based on small numbers of parasites, have been given previously by other workers, including Braun (1900*a*, *b*), Odhner, (1905), Nybelin (1933) and Hopkins (1934), a fuller description is given in the present paper. A complete description of *C. farionis* is thought to be unnecessary, in view of the numerous descriptions and figures of this species given in the literature (Olsson, 1876; Blanchard, 1891; Looss, 1902; Nicoll, 1909; Brown, 1927; Hopkins, 1934); but this species is compared briefly with *C. metoecus*.

(*a*) *Taxonomy of* Crepidostomum metoecus (*Figs.* 3, 5–7)

The body is roughly oval in outline, with a somewhat pointed posterior end and a narrow neck region. It measures from 0·87 to 2·20 mm. in length and from 0·16 to 0·33 mm. at the broadest point, which occurs at the level of the ovary. The oral sucker is subterminal and almost globular in shape. It measures between 0·15 and 0·23 mm. in diameter. The ventral sucker, which occurs in the anterior third of the body, is only slightly larger than the oral sucker. It measures from 0·19 by 0·20 mm. to 0·28 by 0·26 mm. The ratio of the oral sucker to the ventral sucker varies from 1:1·09 to 1:1·26. Six oral papillae, measuring from 0·0625 to 0·070 mm. in length and 0·0375 mm. in breadth, arise from the muscular portion of the oral sucker. They are nearly equal in size and occur in the same transverse plane, occasionally projecting beyond the anterior level of the body. The forebody is highly mobile in living specimens and in the antero-dorsal region lateral to the pharynx there are numerous small scattered granules of pigment.

The oral cavity leads into a very short prepharynx. The pharynx is rounded or ellipsoidal in shape. It measures from 0·06 to 0·09 mm. in length and is never more than half of the length of the oral sucker. Following the pharynx is a sinuous oesophagus, approximately equal in length to the pharynx. The gut bifurcation occurs midway between the oral and ventral suckers. The gut caeca are from 0·030 to 0·035 mm. broad and pursue a lateral course towards the posterior extremity. They converge towards one another behind the posterior testis and terminate a short distance beyond the half-way point, between the posterior testis and the posterior extremity.

The excretory bladder is a median, dorsal, cylindrical sac, extending from the subterminal, dorsally situated excretory pore to the level of the anterior third of the anterior testis. Here it bifurcates to form two lateral excretory ducts.

The common genital pore occurs in the median line, a short distance behind the gut bifurcation and it communicates with a shallow genital atrium. The testes are paired, tandem structures lying close together behind the ovary and the receptaculum seminis. The intertesticular space is sometimes absent. In shape they vary from a compressed oval to an elongated ellipsoid. The anterior testis measures from 0·13 by 0·23 mm. to 0·31 by 0·35 mm. and it is usually broader than long. The posterior testis, on the other hand, tends to be longer than broad. It measures from

0·23 by 0·18 mm. to 0·38 by 0·24 mm. Both vasa efferentia arise from the antero-dorsal surfaces of their respective testes and unite in the ovarian region to form a short vas deferens which enters the cirrus sac. The cirrus sac is an elongated, club-shaped muscular structure situated dorsally to the ventral sucker and extending anteriorly to the level of the genital atrium and posteriorly as far as the middle of

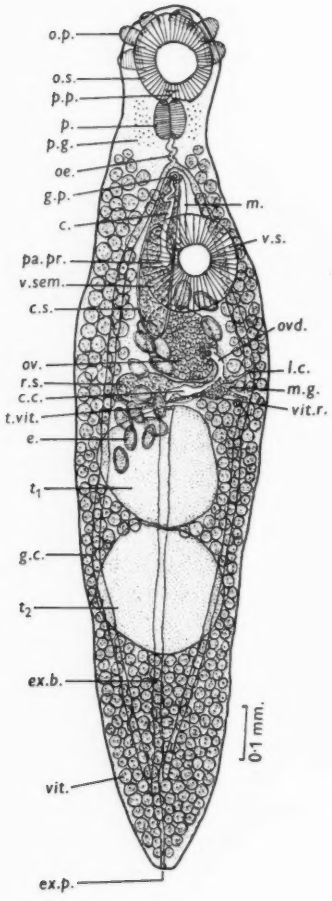


Fig. 3

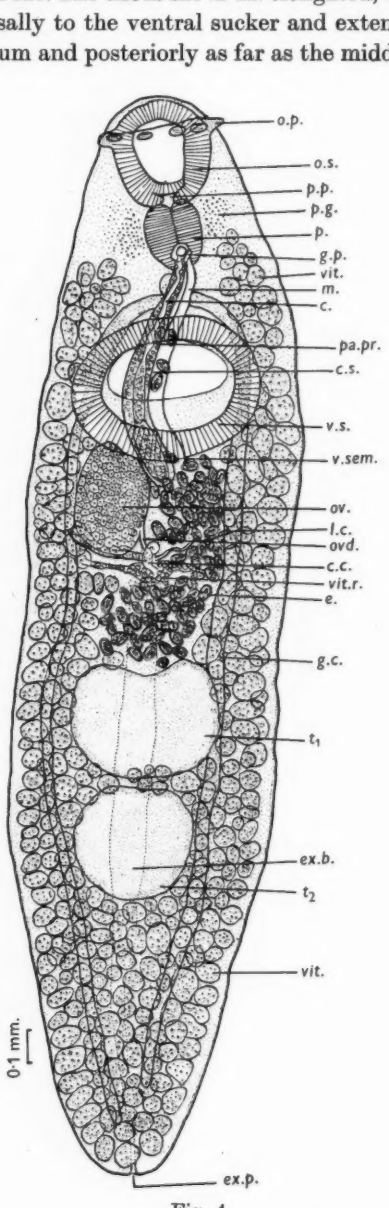


Fig. 4

Fig. 3. *C. metoecus* (Braun), ventral view.
Fig. 4. *C. farionis* (Müller), ventral view.

the ovary. It lies slightly to the left or to the right of the median line. Within the cirrus sac the vas deferens enlarges to form the large, bipartite vesicula seminalis, which occupies the posterior two-thirds of the sac. The posterior chamber of the vesicula seminalis is considerably larger and overlaps the small ventrally situated anterior chamber. The latter communicates at its posterior extremity with the ovoid pars prostatica. Continuous with the pars prostatica is the narrow, muscular,

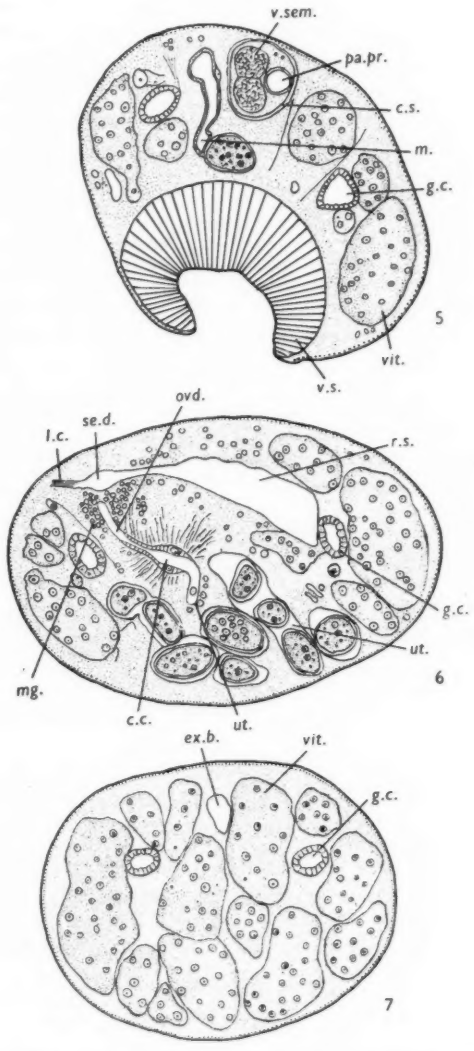


Fig. 5. Transverse section through the ventral sucker of *C. metoecus*.
Fig. 6. Transverse section through the ootype of *C. metoecus*.
Fig. 7. Transverse section through the post-testicular region of *C. metoecus*.

unarmed, eversible ejaculatory duct. It occurs in the narrow anterior part of the cirrus pouch and is surrounded by prostatic cells.

The ovary lies slightly to the left or right of the median line, in the dorsal field between the ventral sucker and the receptaculum seminis. It is an almost globular structure, measuring from 0·14 by 0·14 mm. to 0·24 by 0·28 mm. The oviduct arises dorsally from the inner border of the posterior third of the ovary. It is a short structure which loops ventrally towards the median line, behind the ovary, to form the central chamber of Mehlis's gland, situated beneath the receptaculum seminis. The receptaculum seminis is a large, pyriform structure, measuring between 0·7 and 0·9 mm. in length and extending transversely across the body behind the ovary. At its narrow, distal end it dilates dorsally to form a small, globular swelling from which the short seminal duct to the oviduct and Laurer's canal take their origin. Laurer's canal is short and pursues an almost straight course laterally to open dorsally above the gut caecum on the side opposite the broad end of the receptaculum seminis. Continuous with the central chamber and emerging ventrally is the uterus. The uterine loops which occur beneath the ovary, receptaculum seminis and the anterior testis are few in number. The metraterm is a short, muscular duct extending from the middle of the ventral sucker and turning ventrally to open into the genital atrium. The oval, intra-uterine eggs are few in number. Usually they number less than thirty, but as many as sixty have been recorded in one specimen. They measure from 0·0625 by 0·0375 mm. to 0·0800 by 0·0375 mm.

The yolk glands are well developed, extending from the posterior level of the pharynx to the posterior extremity of the body. In the pretesticular region they are restricted to the lateral fields, but behind the testes they also extend into the median field. Transverse sections show that they may occur both dorsally and ventrally to the gut caeca in all regions of the body. The two lateral, longitudinal yolk ducts are indistinct. They join one another behind the posterior testis and also immediately behind the ovary by means of a transverse yolk duct in each case. The anterior transverse duct enlarges in the median field to form the vitelline reservoir which is situated ventrally to the posterior region of the receptaculum seminis. The median yolk duct arises from the reservoir and opens into the oviduct a short distance behind the opening of the seminal duct.

(*b*) *Comparison between* Crepidostomum metoecus *and* C. farionis

A comparison between Figs. 4, 8, 9 and 10, representing *C. farionis*, and Figs. 3, 5, 6 and 7, representing *C. metoecus*, shows that the two species differ from one another in certain fundamental characters. These differences are tabulated below (Table 5).

Geographical distribution of Crepidostomum metoecus *and* C. farionis

Both *C. metoecus* and *C. farionis* are here reported for the first time from three rivers in West Wales, namely the Teify, Rheidol and Pysgotwr. *C. metoecus* is recorded for the first time in Britain.

Degree of infestation and pathogenicity

The data presented in Tables 1, 3 and 4 show that a large proportion of the trout and salmon parr were infested, particularly during the winter. The infestations in individual fish were also high at this time (Tables 1, 3 and 4 and Fig. 2). The highest average number of parasites per fish in the monthly samples investigated

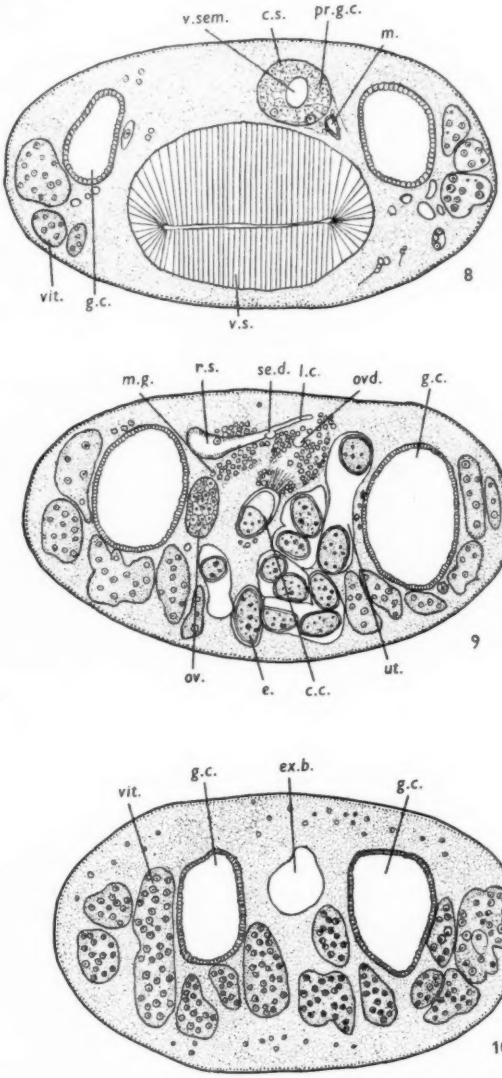


Fig. 8. Transverse section through the ventral sucker of *C. farionis*.
Fig. 9. Transverse section through the ootype of *C. farionis*.
Fig. 10. Transverse section through the post-testicular region of *C. farionis*.

was 70·5 at station A, 83·0 at B, 30·2 at C and 103 at D. The maximum number of parasites found in individual fish was 77 at station A, 157 at B, 133 at C and 122 at D.

No lesions attributable to the *Crepidostomum* species were seen in the intestine of the salmonid fishes examined, and it would appear that, although they occur in such large numbers in the pyloric caeca and the intestine, they are non-pathogenic.

Table 5. *The differences between* Crepidostomum metoecus *and* C. farionis

Character	*C. metoecus*	*C. farionis*
Total length	0·87–2·12 mm.	1·52–5·02 mm.
Neck region	Narrow	No distinct narrowing
Ratio of oral to ventral sucker	1:1·09 to 1:1·26 mm.	1:1·4 to 1:1·5 mm.
Oral papillae	0·062–0·070 mm.	0·037–0·050 mm.
Pharynx	Less than half length of oral sucker	More than half length of oral sucker
Gut caeca	Narrow and short	Wide and long
Excretory bladder	Ends near anterior level of testes	Extends in front of testes
Genital pore	Behind gut bifurcation	In front of gut bifurcation
Vesicula seminalis	Chambers overlapping	Chambers arranged in straight line
Vitelline follicles	Small, occur dorsally and ventrally to gut caeca	Large, occur ventrally to gut caeca
Space between ovary and anterior testis	Narrow	Wide
Number of eggs	Rarely more than 30	Up to 100
Size of eggs	0·062 by 0·037 mm. to 0·080 by 0·037 mm.	0·065 to 0·045 mm. to 0·087 by 0·042 mm.

Host specificity and immunity

Both species of *Crepidostomum* are recorded from the salmon parr and eels for the first time in the present study. In both of these host fish, however, the degree of infestation was appreciably less than it was in the trout. Thus only one eel out of the 243 examined harboured the parasite. Table 3 shows that the salmon parr at station B, which were in their first and second years, were appreciably less heavily infested than trout of the same age, the difference being significant at the level of 5% probability.

The relationship between the degree of infestation and the age of the host was examined as a measure of the possible development of age resistance, either natural or of an acquired nature. Table 3 and Fig. 2 show that at station B there is an increase in infestation with increase in the age of the host. The differences in the degree of infestation between the age groups is significant to a level of 0·1%. A similar trend is shown at the other stations (Table 4).

Seasonal variation in the numbers of parasites

Table 3 and Fig. 2, based on the results from station B, reveal that the degree of infestation is at a maximum during the winter and at a minimum during the summer months. The seasonal differences are significant to a level of 0·1% and a

similar trend is apparent at stations A, C and D (Table 4). A consideration of the rise in the degree of infestation in the autumn, following the decline of the summer months, indicates that the older fish are becoming infested more rapidly than younger fish.

Life cycle

Brown (1927) found the early stages of *C. farionis* inhabiting the freshwater clams, *Pisidium* sp. or *Sphaerium corneum*. The ophthalmoxiphidiocercariae encyst in the mayfly *Ephemera danica* according to Brown (1927) and in *Gammarus pulex* according to Baylis (1931). During the present investigation *Pisidium* species were found to occur at all four stations and *Sphaerium corneum* at stations A and B on the Teify. An examination of 412 *Sphaerium corneum* from station A, during July and August, revealed that only one clam contained mature rediae that were releasing ophthalmoxiphidiocercariae of the kind described by Brown (1927). Of the known intermediate host only one is known to occur at the stations examined, namely *Gammarus pulex* at stations A and B. No other second intermediate hosts were discovered, although several hundred arthropods were examined under a dissecting microscope.

IV. DISCUSSION

Double infestation of trout and salmon parr with Crepidostomum metoecus *and* C. farionis

Corbett (1955) noted the double infestation of trout with these two species of trematode, but did not mention a possible habitat isolation. The observations here recorded are comparable with those of Baer (1952), who gives instances of double infestation involving closely allied tapeworms, in which the different species were invariably found to be inhabiting different regions of the gut.

Although it would seem that these two species of *Crepidostomum* show some degree of habitat isolation, in view of their close proximity and the absence of hybrids, the assumption must be made that they are incompatible. It is probable that geographic isolation has preceded habitat isolation, so that speciation became possible. The seasonal fluctuations in the numbers of these two species show that they are comparatively short-lived, and this fact would contribute towards their geographic isolation, making it less easy for migratory salmonid fish to disseminate the parasite. The suggestion, made by Hopkins (1934), that progenetic metacercariae carried by mayflies, or by accidental hosts such as bats, may assist in breaking down geographic barriers, has yet to be proved.

Geographical distribution of C. metoecus *and* C. farionis

The geographical distribution of *C. farionis* is known to be very widespread in the northern hemisphere. It has previously been recorded from the British Isles by Nicoll (1909, 1924), Southern (1912), Brown (1927), Baylis (1928, 1939), Rawson (1952), Corbett (1955) and from France, Austria, Germany, Denmark, Sweden, Finland, Canada, Alaska and the United States (Hopkins, 1934).

C. metoecus, on the other hand, appears to have a more restricted distribution. It has previously been recorded only in Sweden (=*C. suecicum* Nybelin, 1933),

Austria (Braun, 1900*a*) and Ireland (Corbett, 1955). The present record is, therefore, new to Britain.

The geographical distribution of both *C. farionis* and *C. metoecus* appears to be governed by that of the Salmonidae and the first intermediate host, which is a clam of either the genus *Sphaerium* or *Pisidium*. Hopkins (1934) has suggested that, in view of the tremendous geographical range of *C. farionis*, it is probable that this trematode must be able to adapt itself to invertebrate second intermediate hosts, in addition to those already known, in different parts of its range, although it probably remains dependent on Sphaeriidae for its molluscan host. These suggestions are supported by the findings of the present investigation. Thus Sphaeriidae occur at all four stations, whereas the only known intermediate host found to occur at the stations examined was the sparsely distributed *Gammarus pulex* at stations A and B. It is apparent, therefore, that *C. farionis* must have a hitherto undiscovered second intermediate host.

The life history of *C. metoecus* has not been made the subject of a detailed study, but Noller (1928) has postulated that the larval stage is *Cercaria arhopalocerca* from a *Pisidium* species. This is reported to encyst in Chironomid larvae of the genus *Chironomus* or *Corethra*.

The degree of infestation and pathogenicity

It has been shown that the fishes examined in the present study commonly contained many trematodes, but little information of a comparable nature is available from other sources. Rawson (1952) never found more than two worms in each of the fourteen trout examined by her, while Corbett (1955) recovered 118 *Crepidostomum* specimens from eight trout examined by her in Ireland during April and May. In view of the inadequacy of comparable data, no comment can be made concerning the present level of infestation.

Digenetic trematodes within the intestine are rarely pathogenic as shown by their independence from serological reaction (Dawes, 1946; Baer, 1952). A few, however, such as *Fasciolopsis* species, produce a profound toxaemia, while others such as *Heterophyes* and *Metagonimus* species may cause damage to the gut wall. The *Crepidostomum* species studied do no apparent damage to the host and appear to feed on mucus.

Host specificity and immunity

Crepidostomum farionis is not closely specific judging from the fact that it has been recorded from a variety of freshwater fish, including *Salmo* spp., *Salvelinus* spp., *Coregonus* spp., *Thymallus thymallus*, *Stenodes leucichthys* and *Perca fluviatilis* (Hopkins, 1934). *C. metoecus* has likewise been recorded from Salmonidae such as *Salmo* spp. and *Thymallus thymallus*, and also from *Lota lota*, *Cottus gobio*, *Perca fluviatilis*, and the bats *Nyctalus maximus* (Fatio) and *N. noctula* (Schreber). Hopkins (1934) has suggested that bats are occasional or accidental hosts of *C. metoecus*, in view of the wide differences between the hosts and the fact that the parasites have only once been recorded from bats.

It appears from the present investigation that both species of *Crepidostomum*

show some measure of specificity. As only one eel out of the 243 examined harboured these helminth parasites, it may be assumed that this was almost certainly an accidental infestation, caused by the eel ingesting an infested Salmonid. It is well known that the eel is piscivorous and at stations A and B, 25·9 and 23·9 % respectively of the eels had preyed upon fish. Both the eel and the trout subsist, in the main, on similar aquatic organisms (Thomas, 1954), including one of the known intermediate hosts of *C. farionis*, namely, *Gammarus pulex*. It would appear, therefore, that the eel is physiologically immune to both species of *Crepidostomum*.

Table 3 shows that the salmon parr at station B, which were in their first or second year, were appreciably less heavily infested than trout of the same age, the differences being significant at the level of 5 % probability. This difference may be attributed to a partial physiological or ecological immunity on the part of the salmon parr. Thomas (1957) has shown by a detailed study that, although the two salmonid fishes eat essentially the same food organisms, there are quantitative differences in their consumption of these. It is possible, therefore, that the salmon parr ingest fewer of the second intermediate hosts of *Crepidostomum* species than the trout do. It was found, for instance, that 6·6 % of the trout examined at station B had preyed on *Gammarus pulex* compared with only 3·3 % of the salmon parr.

Host species may develop immunity as a result of acquired immunity; or as a result of premunition which prevents superinfection; or as a result of an increased natural resistance, namely age resistance (Taliaferro, 1940). It is evident that age resistance is not readily differentiated from the first two possibilities. The rise in the degree of infestation in the autumn, following the decline in the summer months, shown by all age groups, strongly suggests that the trout had not acquired an immunity as a result of previous infestations; nor is there any immediate indication of premunition. It is possible that the high infestation during the winter is controlled by premunition. Baer (1952) has suggested that there is a definite relationship between the size of the gut and the number of helminth parasites, the number of parasites being controlled by the amount of available food supply and not by an immunological reaction.

Age resistance, however acquired, would result in fewer parasites occurring in the older fish. Present results show that there is an increased rate of infestation with advancement of age. It is clear, therefore, that total immunity, whether through age or acquired, never develops. The fact that the older fish are more heavily infested is readily explained on the premise that the larger fish require more food organisms to satisfy their needs than do the younger fish, and consequently are more liable to a greater number of infested intermediate hosts.

Previous workers, such as Essex & Hunter (1926) and Bangham (1944), have also reported heavier intestinal infestations of helminth parasites in older fish. Frankland (1955), working with the monogenetic trematode, *Diclidophora denticulata* also found this. There is no support for the suggestion made by Rawson (1952) that *C. farionis* is only found in immature fish. Cameron (1934) and other writers are of the opinion that it is doubtful whether the host shows age resistance to its own

species of parasite. The absence of any evidence of age resistance in the present studies probably indicates an old and stable relationship between the *Crepidostomum* species and the trout.

Seasonal variation in the degree of infestation

There are three possible reasons for the decline in the extent of infestation during the summer months. (1) It may be a non-specific immunity caused by the presence of large numbers of other intestinal parasites, such as the Acanthocephalan *Neoechynorhynchus rutili* and the nematode *Cucullanus truttae*, but this is not the case as there is no significant increase in the numbers of these parasites during the summer (Thomas, 1954). (2) Earlier infestations built up during the winter months might have resulted in premunition, but, as the reaction is apparently not a humoral one, it is improbable that it would result in such an abrupt decline in the number of parasites. (3) It has been shown that very few cercariae are produced during July and August. The periodicity of the trematode in the definitive host may therefore be governed by an external factor, namely, the time of swarming of the cercariae, which is in its turn governed by the life cycle of the clam. Brown (1927) also reported a low percentage of infected sphaeriid molluscs examined by him from the River Wharfe during July, August and September.

V. SUMMARY

1. *Crepidostomum farionis* (Müller, 1784) and *C. metoecus* (Braun, 1900) were found to occur together in the same host fish, *Salmo trutta* L., *S. salar* L. and *Anguilla anguilla* (L.) in mid-Wales. It is believed that the latter species constituted an accidental host.

2. *C. metoecus* is recorded in Britain for the first time.

3. There was some evidence of a habitat isolation in individual fish as *C. metoecus* occurred predominantly within the pyloric caeca, while *C. farionis* usually occupied a more posterior station in the intestine.

4. A detailed description is given of *C. metoecus* and the salient features of this species are compared with those of *C. farionis*.

5. The two species of *Crepidostomum* commonly occurred in large numbers in their fish hosts, individual fish harbouring up to 157 worms. No lesions attributable to *Crepidostomum* were, however, detected and there was no evidence of pathogenicity.

6. It would appear that the eel is physiologically immune to both species of *Crepidostomum* and that the salmon parr is partially resistant.

7. The degree of infestation of trout and parr with both the *Crepidostomum* species is at a maximum during the winter months and at a minimum in the summer months. This seasonal variation can be attributed to a periodicity in the swarming of the cercariae.

8. There is no evidence of age resistance or acquired immunity of the trout to infestation by *Crepidostomum* species.

REFERENCES

Baer, J. G. (1952). *Ecology of Animal Parasites.* Urbana: University of Illinois Press.

Bangham, R. V. (1944). Parasites of North Wisconsin fish. *Trans. Wis. Acad. Sci. Arts Lett.* **36**, 291–325.

Baylis, H. A. (1928). Records of some parasitic worms from British vertebrates. *Ann. Mag. Nat. Hist.* (10), **1**, 329–43.

Baylis, H. A. (1931). *Gammarus pulex* as an intermediate host for trout parasites. *Ann. Mag. Nat. Hist.* (10), **7**, 431–5.

Baylis, H. A. (1939). Further records of parasitic worms from British vertebrates. *Ann. Mag. Nat. Hist.* (11), **4**, 473–98.

Blanchard, R. (1891). Notices helminthologiques (2me série). *Mém. Soc. Zool. Fr.* **4**, 420–9.

Braun, M. (1900*a*). Einige Bemerkungen über die Fascioliden der Chiroptera. *Zool. Anz.* **23**, 387–91.

Braun, M. (1900*b*). Trematoden der Chiroptera. *Ann. naturh.* (*Mus.*) *Hofmus., Wien*, **15**, 217–36.

Brown, F. J. (1927). On *Crepidostomum farionis* Müll. (=*Stephanophiala laureata* Zeder), a distome parasite of the trout and grayling. 1. The life history. *Parasitology*, **19**, 86–99.

Cameron, T. W. M. (1934). *The Internal Parasites of Domestic Animals.* London.

van Cleave, H. J. & Müller, J. F. (1934). Parasites of Oneida Lake fishes. Part 3. A biological and ecological survey of the worm parasites. *Roosevelt Wild Life Ann.* **3**, 1–334.

Corbett, M. P. (1955). Occurrence of two species of *Crepidostomum* in brown trout (*Salmo trutta* L.) from North-East Ireland with special reference to *Crepidostomum metoecus* Braun 1900. *Parasitology*, **45**, 186–8.

Crawford, W. W. (1943). Colorado trematode Studies. I. A further contribution to the life history of *Crepidostomum farionis* (Müller). *J. Parasit.* **29**, 379–84.

Dawes, B. (1946). *The Trematoda.* Cambridge University Press.

Essex, H. E. & Hunter, G. W. (1926). A biological study of fish parasites from the central states. *Trans. Ill. Acad. Sci.* **19**, 151–81.

Frankland, H. M. T. (1955). The life history and bionomics of *Diclidophora denticulata. Parasitology*, **45**, 313–51.

Hopkins, S. H. (1934). The Papillose Allocreadiidae. *Illinois Biol. Monogr.* **13**, 1–80.

Looss, A. (1902). Über neue und bekannte Trematoden aus Seeschildkröten. *Zool. Jber.* **16**, 411–889.

Nicoll, W. (1909). Studies on the structure and classification of the digenetic trematodes. *Quart. J. Micr. Sci.* **53**, 391–487.

Nicoll, W. (1924). A reference list of the trematode parasites of British freshwater fishes. *Parasitology*, **16**, 127–44.

Nöller, W. (1928). Zu welchem Trematoden gehört *Cercaria arhopalocerca* Nöller 1925. *S.B. Ges. naturf. Fr. Berl.* 1927, **8–10**, 162–4.

Nybelin, O. (1933). *Crepidostomum suecicum* n.sp., ein Trematode mit ungewöhnlich weiter morphologischer Variationsbreite. *Ark. Zool.* **25**B (1), 1–6.

Odhner, T. (1905). Die Trematoden des arktischen Gebietes. *Fauna arct., Jena*, **4**, 291–372.

Olsson, P. (1876). Bidrag till skandinaviens helminthfauna. *K. svenska VetenskAkad. Handl.* **14**, 1–35.

Pearse, A. S. (1924). Observations on parasitic worms from Wisconsin fishes. *Trans. Wis. Acad. Sci. Arts Lett.* **21**, 147–160.

Rawson, D. (1952). The occurrence of parasitic worms in British freshwater fishes. *Ann. Mag. Nat. Hist.* (12), **5**, 877–87.

Southern, R. (1912). Clare Island Survey, pt. 56, Platyhelmia. *Proc. R. Irish Acad.* **31**, 17.

Taliaferro, W. H. (1940). The mechanism of acquired immunity in infections with parasitic worms. *Physiol. Rev.* **20**, 469–92.

Thomas, J. D. (1954). The food, growth and helminth parasites of trout (*Salmo trutta* L.) from four contrasted habitats with some similar observations on the salmon parr (*Salmo salar* L.) and eels (*Anguilla anguilla* (L.)). Ph.D. Thesis, University College of Wales, Aberystwyth.

KEY TO LETTERING OF FIGURES

c.	cirrus	*p.*	pharynx
c.c.	central chamber, Mehlis's gland	*p.g.*	pigment granules
c.s.	cirrus sac	*p.p.*	prepharynx
e.	eggs in uterus	*pa.pr.*	pars prostatica
ex.b.	excretory bladder	*pr.g.c.*	prostatic gland cells
ex.p.	excretory pore	*r.s.*	receptaculum seminis
g.c.	gut caecum	*se.d.*	seminal duct from receptacle
g.p.	genital pore	t_1	anterior testis
l.c.	Laurer's canal	t_2	posterior testis
m.	metraterm	*t.vit.*	transverse vitelline duct
m.g.	Mehlis's gland	*ut.*	uterus
oe.	oesophagus	*v.s.*	ventral sucker
o.p.	oral papillae	*v.sem.*	vesicula seminalis
o.s.	oral sucker	*vit.*	vitelline follicles
ov.	ovary	*vit.r.*	vitelline reservoir
ovd.	oviduct		

(*MS. received for publication* 19. VI. 1957.—Ed.)

THE SHEEP KED, *MELOPHAGUS OVINUS* L.

I. DISSEMINATION POTENTIAL

By J. H. TETLEY

Massey Agricultural College, Palmerston North, New Zealand

CONTENTS

		PAGE
I.	Introduction	353
	(*a*) Host range	353
	(*b*) Distribution and behaviour on sheep	354
	(*c*) Flocking of sheep	355
	(*d*) Nature of the fleece	355
	(*e*) Young lamb	355
	(*f*) Weaned lamb	356
	(*g*) Lactating ewe	356
	(*h*) Ram	356
II.	Experimental sheep	356
III.	Experiment I. Ked dissemination potential on two sheep	357
IV.	Experiment II. Transfer of keds from a sheep with a high dissemination potential	360
V.	Experiment III. Keds acquired by a sheep with a high dissemination potential	361
VI.	Summary	363
	References	363

I. INTRODUCTION

Some aspects of the dissemination of the sheep ked, *Melophagus ovinus* L., have been studied by Graham & Taylor (1941), MacLeod (1948) and Evans (1950).

For direct dissemination keds require to take up a station on the surface of the fleece from which position they transfer with facility to another sheep if the latter brushes, even momentarily, against them. The term dissemination potential is proposed for the proportion of the population at the fleece surface at a particular point of time. It follows that the rate of transfer of keds depends on the pitch of the dissemination potential, which fluctuates on the one host and differs between hosts, and the frequency of contact of hosts. Fluctuations in the dissemination potential and opportunity for transfer are largely functions of the host pattern. Components of the ked-sheep pattern are defined in the following:

(*a*) *Host range*

The sheep, *Ovis aries*, is the natural host of the ked. Because of the propensity of this parasite to attach to almost any medium that will provide a foothold, other animals can serve as temporary hosts. The infrequency with which sheep have bodily contact with other species explains the few records of such hosts. Working dogs are able to acquire keds directly from sheep, and rabbits, indirectly, by way of the ground. However, on both of these hosts they are short lived. A female ked was found to survive in a muslin cage on a human being for 14 days, imbibing blood and depositing a larva that pupated, though no ked emerged.

(b) Distribution and behaviour on sheep

Graham & Taylor (1941), Macleod (1948) and Evans (1950) have studied the distribution of the ked on the sheep and are in general agreement that the favoured areas of infestation are the under-neck–shoulder region, the sides and the breech. Smaller populations are found on the belly, and few if any keds occur on the back. In the main, the regions of infestation are those which readily come into contact with the fleeces of other sheep. Thus keds on the surface in these areas are nearly always favourably placed for transference to another host.

The balance of populations between the zones of infestation is dynamic. Among young lambs greater numbers of keds are found in the under-neck–shoulder region than in other parts of the fleece. As lambs mature the population becomes more generally distributed throughout the regions mentioned above. MacLeod and Evans, reasoning from differences in populations found in various parts of the fleece at different times, and from the higher ratio of pupae to adults in the under-neck–shoulder region, concluded that there was active migration of keds from the latter area, to which they returned to breed. There is ample evidence that keds will gradually extend their range of infestation, but very little that they will vacate an area by sustained movement that would imply active migration. In their favoured regions of the fleece, over periods of one to a few days, most females did not travel more than a few inches; males tended to move farther. If the klino-kinesis, that keds exhibit when off the sheep, occurs also on the sheep, then a process of gradual extension of the area of infestation may be the normal one.

The data of MacLeod (1948) and Evans (1950) indicate that large numbers of pupae are deposited in regions other than the under-neck–shoulder area, and this the writer also has observed. It seems doubtful, therefore, that special migration for reproductive purposes is a complete explanation of the high pupa-adult ratio in the anterior region. It appears, because there is much to and fro transference of keds from one sheep to another, that it is inevitable there will be dispersion of ked populations over favoured regions of the fleece, giving the appearance of active migration. The pupa-adult ratio in different regions of the fleece could be altered in this way in spite of the finding of Graham & Taylor (1941) that females in advanced age, and presumably of low fecundity, may 'wander aimlessly over the body...'. MacLeod (1948) and Evans (1950) do not mention differences between the sexes in distribution over the sheep. However, there is a tendency for the male–female ratio to be higher towards the posterior end of the sheep's body, which the writer believes is due to the wider ranging habit of the males. There is the possibility that the higher adult–pupa ratio farther back on the sheep, that MacLeod and Evans found, might have been influenced by this fact.

Within the main regions of infestation, and at any one point of time, keds orientate themselves in depth in the fleece. Many will be found at the skin surface, a portion of which will be feeding. The remainder are arrayed at various depths in the fleece including the surface. On full-fleeced heavily infested sheep distinct zones discoloured with ked faeces are to be found just below the fleece surface, which indicates that keds spend a prolonged time there digesting food.

Graham & Taylor (1941) found that keds in the incubator at a temperature of 37° C. did not survive for more than 24 hr. The present work has confirmed this, and has further shown that keds, confined in muslin cages sewn to the skin of sheep, and denied opportunity to migrate to the fleece surface, did not live long. The conclusion is that keds are unable to survive for long periods at the skin on full-fleeced sheep, and at intervals require to take up a station near the fleece surface. This degree of inadaptation to near blood temperature, with its resultant influence on ked behaviour, thus stands the parasite in good stead in providing a mechanism for dissemination.

(*c*) *Flocking of sheep*

Since the ked is able to transfer from one sheep to another during momentary contact, the flocking habit of its host is of considerable significance to the parasite. The brisk movement of sheep, that is frequently the accompaniment of flocking, causes many keds to come to the fleece surface, particularly those in the digestion zone mentioned above. Thus behaviour of host and parasite are correlated in ensuring rapid dissemination. There can be little doubt that the times of general flocking of sheep during the summer, when keds come to the fleece surface in greatest numbers, are the times of most rapid dissemination of the parasite.

(*d*) *Nature of the fleece*

Sheep with dense or cotted fleeces are less prone to have many keds than those with open, better ventilated wool. In New Zealand few keds are found on sheep whose wool is matted with the burrs of Piripiri (*Acaena* sp.). Other things being equal, they are less numerous in very greasy fleeces than in those in which the secretions are in smaller amount.

Because of the numerous bristles over the body, the ked, in greasy fleeces, acquires a thick film of the secretions that must interfere with respiration. As wool on lambs increases in length, there is an accompanying change in the microclimate associated with which keds increasingly appear on the fleece surface. It is a matter for surmise what role changes in fibre type of the wool, the nature of the secretions, blood and the skin have in ked ecology.

(*e*) *Young lamb*

The young lamb has frequent contact with its mother, less with other lambs and still less with other sheep. Most keds are acquired by the lamb from the mother during feeding, and mainly at the anterior part of its body. These move downwards to more sheltered parts of the fleece. Thus it comes about that many accumulate in the under-neck–shoulder region, the first favourable region for infestation that they encounter. Others, in smaller numbers, are to be found on the sides, belly, breech and crutch regions.

For the first few weeks of the lamb's life, while the wool is short, keds apply themselves closely to the skin and few are to be seen on the fleece surface. Until

the wool grows longer and migrations take place to the surface, there is little opportunity for them to return to the mother.

Pupae, in small numbers, are to be found in the more sheltered parts of the fleece. One of these places, the crutch, ceases to be favoured in older sheep.

(*f*) *Weaned lamb*

With transition from a milk to a pasture diet, frequency of contact of the lamb with the mother decreases until, at weaning, complete independence is achieved. At this stage, when the wool is a few inches long, keds are often seen on the fleece surface, and the process of ked exchange, characteristic of mature sheep, is established. The greatest numbers of keds are still to be found in the under-neck–shoulder region, though numbers on the sides and breech are likely to be greater than formerly.

(*g*) *Lactating ewe*

Large numbers of keds are able to survive on at least some full-fleeced, lactating, Romney ewes. The flocking instinct tends to be slightly relaxed, as the shepherds' expression, 'hard to drive', indicates. There is considerably more contact with their own lambs than with other members of the flock. Keds may be seen frequently on the fleece surface, the sides being as well favoured as the under-neck–shoulder region. During this stage, the ewe, lamb and ked comprise an almost closed system, as far as dissemination of the parasite is concerned.

(*h*) *Ram*

During the short tupping season a ram may come into bodily contact with many ewes, so that the significance of the ram as a vector of keds is obvious. Out of season rams pay little attention to ewes and their role as transmitters of keds to ewes at this time diminishes. Both in and out of season, among fighting rams, there is a frequent opportunity for the dissemination of keds.

In the following experiments aspects of the direct dissemination of the sheep ked have been studied with especial reference to dissemination potential.

II. EXPERIMENTAL SHEEP

Two sheep were used in the three experiments.

Sheep 1. Breed: Romney cross. Sex: male. Age: 2 years. Wool: long, fairly coarse, heavily conditioned, cotted.

Sheep 2. Breed: Southdown cross. Sex: male. Age: 8 months. Wool: shorter, finer, less heavily conditioned and better ventilated than that of sheep 1. Uncotted.

Other sheep, referred to incidentally, were a Romney cross.

Sheep 1 and 2 were used in other ked experiments before the present series, in the course of which numbers of keds were transferred to and fro. Thus the initial populations of the present series of experiments were not entirely the outcome of natural processes. It was evident that keds were able to survive, feed, larviposit,

and to emerge from pupae on both sheep during the period immediately preceding the present experiments.

Throughout the experiments the two rams, which were in the off-breeding season, kept one another's company whether they were separated from, or with other sheep. Their frequent butting of each other resulted in contact between the two being much more frequent than with other sheep.

III. EXPERIMENT I. KED DISSEMINATION POTENTIAL ON TWO SHEEP

For a few hours, on each of 4 days, the keds that came to the fleece surface of the two sheep were removed as they appeared, counted, and their sex determined. The sheep were kept standing quietly in a field pen exposed to the sun. At the close of each day's observations the remaining keds on the sheep were removed and counted. Finally, each day, the keds were returned to the sheep from which they were taken. Between periods the two experimental sheep grazed with other infested sheep. The results are given in Table 1.

The relatively large initial numbers of keds found on the fleece surface on each date included some which had been there for an unknown time. On other sheep, in some instances, keds were seen to remain on the surface for at least 40 min. While this fact complicates correlation of the data with length of period, the results remain an indication, for particular dates, of differences in ked behaviour between the two sheep.

It is shown in Table 1 that between 10·2 and 44·7 % of the ked population came to the fleece surface of one or other of the sheep during the daily periods. That is, the dissemination potentials were such that a large proportion of the keds could have transferred to another host in a few hours. In Table 2 it is shown, among a miscellany of sheep on various dates, that up to 28·4 % of the ked population were present on the surface at particular points of time. This indicates that a high dissemination potential is not uncommon.

On sheep 1 respectively 44·7, 16·6, 37·5 and 29·9 % of the population appeared on the fleece surface on the four dates, compared with the lower percentages of 30·6, 10·2, 21·3 and 15·9 on sheep 2. This means that, while keds were able to survive, larviposit and feed on both sheep, their behaviour on sheep 1 resulted in a greater proportion taking up a station on the fleece surface favourable for their transfer to another host.

Associated with difference in the dissemination potential between the two sheep, there was a corresponding trend in their populations. On sheep 1 the initial number of 134 fell to 87 at the last date, whereas, on sheep 2 the respective figures were 108 and 107. The intervening fluctuations did not closely parallel difference in dissemination potential, though it is notable for sheep 1 that the highest percentage loss, 55 % between dates 3 and 4 September, was considerably greater than the highest for sheep 2, 21 %, between 5 and 7 September. Since fluctuations in population are the resultant of keds acquired and those lost, and the number of these is dependent on opportunities for transfer, it would not be surprising if day-to-day population changes were not always correlated with differences in dissemination potential.

Table 1. *Keds appearing on the surface of the fleeces of two sheep*

Period	Sheep no. 1 Males	Sheep no. 1 Females	Sheep no. 1 Total	Sheep no. 2 Males	Sheep no. 2 Females	Sheep no. 2 Total
September 3. Weather mild, sunny until 1.30 p.m.; following which the day was mainly overcast; bright sunshine 7·1 hr.; maximum temperature in shade 55·4° F.; slight wind; rainfall nil; fleeces dry.						
*12.50–1.00 p.m.	15	26	41	8	6	14
* 1.00–1.30 p.m.	5	10	15	12	4	16
1.30–2.00 p.m.	1	3	4	2	1	3
2.00–4.00 p.m.	0	0	0	0	0	0
Total from fleece surface	21	39	60	22	11	33
Sex ratio (%)	35·0	65·0	—	66·6	33·3	—
Total on sheep	51	83	134	69	39	108
Sex ratio (%)	38·1	61·9	—	63·9	36·1	—
Percentage on fleece surface	41·1	46·9	44·7	32·9	28·2	30·6
September 4. Weather mild, overcast; bright sunshine 3·2 hr.; maximum temperature 57·5° F.; light wind; rainfall, a trace; fleeces dry.						
1·50–2.00 p.m.	2	4	6	3	3	6
2.00–2·30 p.m.	1	2	3	1	0	1
2.30–3.00 p.m.	1	0	1	2	1	3
Total from fleece surface	4	6	10	6	4	10
Sex ratio (%)	40·0	60·0	—	60·0	40·9	—
Total on sheep	23	37	60	61	37	98
Sex ratio (%)	38·3	61·7	—	62·2	37·8	—
Percentage on fleece surface	17·4	16·2	16·6	9·8	10·8	10·2
September 5. Weather mild, cloudy intervals; bright sunshine 9 hr.; maximum temperature 60·4° F.; strong wind; rainfall nil; fleeces dry.						
* 1.00–1.10 p.m.	5	8	13	2	2	4
* 1.10–1.30 p.m.	1	2	3	1	1	2
* 1.30–2.00 p.m.	5	8	13	2	4	6
2.00–2.30 p.m.	0	1	1	0	2	2
* 2.30–3.00 p.m.	1	0	1	6	4	10
* 3.00–3.30 p.m.	1	1	2	4	1	5
Total from fleece surface	13	20	33	15	14	29
Sex ratio (%)	39·5	60·5	—	51·7	48·3	—
Total on sheep	33	55	88	81	55	136
Sex ratio (%)	37·5	62·5	—	59·6	40·4	—
Percentage on fleece surface	39·3	36·4	37·5	18·5	25·4	21·3
September 7. Weather mild, mainly overcast; bright sunshine 2 hr.; maximum temperature 58·2° F.; strong wind; rainfall, a trace; fleeces dry.						
12.45–1.15 p.m.	3	1	4	2	3	5
* 1.15–1.45 p.m.	7	10	17	3	3	6
1.45–2.15 p.m.	1	1	2	1	0	1
2.15–2.45 p.m.	0	1	1	3	0	3
2.45–3.15 p.m.	0	2	2	1	1	2
Total from fleece surface	11	15	26	10	7	17
Sex ratio (%)	42·3	57·7	—	58·8	41·2	—
Total on sheep	35	52	87	62	45	107
Sex ratio (%)	40·2	59·8	—	57·9	42·1	—
Percentage on fleece surface	31·4	28·8	29·9	16·4	15·5	15·9

* Periods during which bright sunshine occurred.

The experiment was carried out in weather of intermittent bright sunshine and cloud. The total of periods of the four dates during which bright sunshine occurred was 3 hr. 10 min., in the course of which 168 keds appeared on the fleece surface of the two sheep, mainly in regions directly exposed to the sun. During the 6 hr. 10 min. of continuous cloud 50 keds appeared. Expressed as hourly rates the respective figures were 50·4 and 8·3. It was apparent that, had it been physically possible to subject the whole region of ked infestation on the sheep simultaneously to direct sun rays considerably larger numbers of keds would have moved to the surface.

Table 2. *Keds found on the fleece surface of miscellaneous sheep*

Sheep no.	Date	Time	Age of sheep	Keds on surface of fleece				Total on sheep		
				Males	Females	Total	Percentage of total	Males	Females	Total
3	19 Sept.	10.15 a.m.	2 yr.	22	35	57	21·6	129	134	263
4	25 June	3 p.m.	10 mth.	7	24	31	15·9	62	132	194
5	3 July	11.15 a.m.	11 mth.	4	15	19	16·5	49	66	115
6	11 Oct.	2.30 p.m.	2 yr.	8	12	20	14·4	58	81	139
7	1 July	12 noon	11 mth.	0	38	38	28·4	51	83	134

During the periods of continuous cloud the dissemination potential on the two sheep was similar, being 3·9 % per hour of the daily average population on sheep 1, compared with 3·7 % for sheep 2. The comparable figures for the periods with bright sunshine were 36·6% and 19·9% respectively. Allowing for a slight reduction in the latter pair of figures, because some keds were already on the surface when observations began, it is clear that bright sunshine had a differential effect on the dissemination potential on the two sheep.

Evans (1950) found, by raising the air temperature, presumably under conditions of uniform intensity of light, that keds could be stimulated to appear on the surface. The present work has shown that the temperature just below the surface of a full fleece in direct sunshine, when the air temperature was 65·0° F., rose to 123·0° F. in a few minutes. When a sheep was held, lying on its side, so that the sun-rays fell approximately at right angles to the area of infestation, keds quickly came to the surface and tended to move away from the sun. By slowly rolling the sheep on its back so that there was time for the temperature to rise, keds were induced to move away from the sun and cross the belly to a point on the other side higher than that at which the experiment began. The conclusion is drawn, in the present experiment, that movement to the fleece surface during periods of bright sunshine was not due to a change in the intensity of light, but was caused by the quickly mounting temperature in regions of infestation directly exposed to the sun. It is further concluded that the difference in the dissemination potential between the two sheep arose from similar degrees of temperature change at the fleece surface superimposed on fleeces of different character and resulting in differences in undetermined factors of the respective microclimates.

IV. EXPERIMENT II. TRANSFER OF KEDS FROM A SHEEP WITH A HIGH DISSEMINATION POTENTIAL

Sheep 1 and 2 were placed in a paddock by themselves on 6 October, the keds on sheep 2 having been removed and added to those already on sheep 1. During the ensuing 5 days the counts were made as shown in Table 3. Keds acquired by sheep 2 were returned to sheep 1 after each count. Pupae were not counted. Keds were found on the surface of sheep 1 as follows: 6 October, prior to supplementing the population of this sheep at 1 p.m., 9 of a total of 21; 10 October, 2·45 p.m., 18, and in the next few moments, 22 more when the sheep moved about; 11 October, 2.30 p.m., 45 keds after activity on the part of the sheep. The dissemination potential on this sheep was therefore high. This experiment immediately followed Expt. III.

Table 3. *Accumulation of keds on sheep*

		Sheep no. 1			Sheep no. 2			
Date	Time	Males	Females	Total	Males	Females	Total	Gain in population per 24 hr.
6 Oct.	1.00 p.m.	139	146	285	0	0	0	—
8 Oct.	4.00 p.m.	—	—	—	13	18	31	14·6
10 Oct.	2.00 p.m.	—	—	—	18	20	38	20·7
11 Oct.	2.30 p.m.	50	69	119	8	12	20	19·6

From Table 3 it can be seen that the population on sheep 2 declined from 285 to 119 in the 5 days of the experiment. The disappearance of keds from this sheep would be in excess of 166 since larvae deposited 21 days before the experiment were giving rise to adults at this time. From Table 4 (Expt. III) it can be seen that such larvae were derived from less than 54 females. As a female deposits one larva at about 7 days interval, not more than a daily average of seven could have been produced. Thus the disappearance of keds from this sheep could not have been more than 166 plus 35. The decline in the population on sheep 2 could have been partly due to keds dying, but few dead individuals were found and it is concluded that this factor was of small significance.

Evans (1950) attributed losses of keds to the feeding of starlings and other birds. No evidence in support of this popular theory has yet been provided from analyses of the crop contents of birds, and the present writer has not made such examinations. In New Zealand starlings may be commonly seen on the backs of sheep and to peck at the wool. Keds, however, are seldom to be found in this region. Starlings pay little attention to those zones of the fleece favoured by keds nor do they appear particularly attracted to heavily infested sheep. The conclusion is that starlings and other birds are not a significant factor in reducing ked populations.

Host predation has been cited by Evans (1950) as of significance in reducing ked numbers. In my own experience heavily infested sheep bite at their wool occasionally, but in general sheep pay little attention to small disturbances on their fleece surface and it is doubtful whether keds in that position could cause sufficient irritation to evoke response. Further, keds in the favoured region anterior to the

shoulder are inaccessible to the biting of sheep. This factor also is not considered to be significant in the present experiment.

Of causes for the decline in the population on sheep 2 there remain loss to the ground and transfer to sheep 1, its single associate. On reference to Table 3 it is immediately apparent that only a minority of the keds leaving sheep 1 transferred to sheep 2. Evans (1950) found that keds 'often lose their hold of the fleece, drop to the ground....'. In the course of the present work this happened once in the field. In laboratory experiments, when the temperature was raised much above normal, keds were induced to release their hold on wool fibres and fall to the floor. However, search of the yards of freezing works through which thousands of ked-infested sheep had been rushed, under conditions that might be considered favourable for the parasite to fall to the ground, was without success, except once, when what possibly could have been a crushed specimen was discovered.

In the light of this experience, it does not seem possible that many keds simply fall to the ground. It is evident from the data on keds at the fleece surface of sheep 1, when up to 45 (38 % of the population) were found at particular points of time, that there was a high dissemination potential on this sheep. Had the data been on a 24 hr. basis, it is probable that the potential would have been found to be much higher. Assuming that the population on sheep 1 had been augmented by a natural increase of 35 during the course of the experiment, it is possible that approximately 200 keds left this sheep in the 5 days. On the basis of a geometrical progression this represents a daily average loss of 17·8 %, which is much lower than the proportion of keds that were known to have been on the fleece surface. That is, all keds on the fleece surface did not leave this sheep. The writer believes that departure of keds from sheep depends on the opportunity provided by contact of keds with some medium to which they can clutch. Twice keds were seen to move from a stationary sheep, one on to grass and the second on to a wooden rail. Search for keds on pasture was without success either by inspection or by dragging a woolly sheepskin slowly over camping grounds.

A total of 89 keds was found on sheep 2, some of which may have been involved in more than one transfer. As pupae on this sheep were giving rise to adults, the number of keds originating from sheep 1 would be correspondingly fewer. The keds that left sheep 1 and were acquired by sheep 2 must have been about 31 % of the initial adult population, an average of 6 % per day, or less than a half of the transfers from sheep 1.

V. EXPERIMENT III. KEDS ACQUIRED BY A SHEEP WITH A HIGH DISSEMINATION POTENTIAL

All the keds on sheep 1 were transferred, on 7 September, to sheep 2. From this date until 3 October the two sheep were allowed to graze with other infested sheep, and then from 3 to 6 October they were placed by themselves in a small paddock. Keds were counted as shown in Table 4.

Keds were seen on the fleece of sheep 1 at particular points of time, as follows: 2 October, 13, 3 October, 30 of the total of 98 on the sheep, 6 October, 9 of the 21 on the sheep. There was no doubt, from the behaviour of the two sheep,

whether others were present or not, that sheep 2 was the main vector of keds for sheep 1 throughout the experiment. Pupae were not counted.

In contrast with what happened in Expt. I, the initial population, this time on sheep 2, increased from 194 to 264 by the end of the experiment. Natural increase contributed to this. In Expt. II keds rapidly disappeared from sheep 1, but, in the present experiment, they were acquired in relatively large numbers by this sheep. Between 7 September and 3 October the population rose to 98, representing an average daily gain, including natural increase, of 3·8 keds. From 3 to 6 October a total of 82 keds was acquired by this sheep, a daily average of 27·3. From the history of this sheep, given in Table 4, natural increase could have accounted only for a small percentage of this total, and the keds must have come almost entirely from sheep 2.

Table 4. *Accumulation of adult keds on sheep*

	Population of keds					
	Sheep no. 1			Sheep no. 2		
	Males	Females	Total	Males	Females	Total
7 Sept.	0	0	0	97	97	194
10 Sept.	—	—	*	99	79	178
15 Sept.	—	—	*	93	84	177
3 Oct.	44	54	98	—	—	*
5 Oct.	24	37	61	—	—	*
6 Oct.	7	14	21	132	132	264

* No examination made.

The high rate of accumulation in the latter short periods of the experiment is explained by keds having had insufficient time to have gained stations favourable for their transfer from this animal. In other experiments it was found that their passage through wool was slow, females moving an average of 4·9 in. and males 10·2 in. from a point of liberation in 2 days. The slow rate of population growth during the period from 7 September to 3 October took place in the presence of a similar pitch of exposure to parasitism from sheep 2 to that which obtained in the later stage of the experiment. If the average rate of keds acquired was the same as in the immediately following short periods of accumulation (3–6 Oct.) and if the average daily rate of loss was the same as in Expt. III (17·8 %) it would be expected, on the basis of a geometrical progression, that the population on this sheep would have grown to 142. Under the circumstances this figure accords well enough with the 98 that were found.

In Expt. III, with concentration of keds initially on sheep 2, the total population on the two sheep increased from 194 to 285, while in Expt. II (immediately afterwards), when keds were all placed on sheep 1 and when, if anything, the natural rate of increase on sheep 2 may have been greater than before, the total population on the two sheep fell from 285 to 139. Thus sheep 1 behaved as a disinfecting agent in the system.

VI. SUMMARY

Components of the intricate ked-sheep pattern, of significance in dissemination of the ked, are defined. It was found that the dissemination potential (percentage of population on the fleece surface) on one sheep was constantly greater than on a second. Bright sunshine caused an increase in the dissemination potential, particularly on the first sheep. It was concluded that the stimulating factor was a rise in the fleece temperature in regions directly exposed to the rays of the sun.

The factor or factors causing differences in dissemination potential arose from similar degrees of temperature change being superimposed on inherently different fleeces. The sexes behaved similarly on both sheep.

Keds were able to transfer readily between the two sheep. However, when they were concentrated on the sheep with a high dissemination potential, the population of the two-sheep system declined rapidly; when they were placed on the sheep with a low dissemination potential, the population of the two-sheep system increased. Approximately half of the keds leaving the sheep with a high dissemination potential did not reach a second host. It thus acted as a disinfecting agent.

Bird and host predation were discounted as factors in causing ked population change.

REFERENCES

Evans, G. O. (1950). Studies on the bionomics of the sheep ked, *Melophagus ovinus* L., in west Wales. *Bull. Ent. Res.* **40**, 459–78.

MacLeod, Y. (1948). The distribution and dynamics of ked population. *Parasitology*, **39**, 61–8.

Graham, N. P. H. & Taylor, K. L. (1941). Studies on some ectoparasites of sheep and their control. I. Observations on the bionomics of sheep ked (*Melophagus ovinus* L.). *Pamphl. Coun. Sci. Industr. Res. Austr.* no. 108, pp. 7, 9–26.

(*MS. received for publication* 18. vii. 1957.—Ed.)

THE SHEEP KED, *MELOPHAGUS OVINUS* L.

II. KEDS ACQUIRED BY A LAMB FROM ITS MOTHER

By J. H. TETLEY

Massey Agricultural College, Palmerston North, New Zealand

(With 5 Figures in the Text)

CONTENTS

		PAGE
I.	Introduction	364
II.	Design of the experiment	364
III.	Habits of the ewe and the lamb	365
IV.	Origin of keds on the lamb	365
V.	Vector potential of the ewe	366
VI.	Populations on the lamb and length of period	367
VII.	Meteorological factors and ked dissemination	370
	(i) Humidity	371
	(ii) Light	371
	(iii) Temperature	372
VIII.	Dissemination index of keds	372
IX.	Population pattern on the lamb	373
X.	Summary	374
	References	374

I. INTRODUCTION

In Part I populations of keds on fully fleeced sheep were studied and the extent of their transfer from one sheep to another was explained in terms of dissemination potential. Dissemination of the ked is now considered in connexion with a second facet of the host pattern, the relation of the lamb to its mother.

II. DESIGN OF THE EXPERIMENT

The rate at which keds were acquired was observed on a spring-born lamb from 3 days of age until it was 42 days old. The mother was full fleeced and heavily infested with keds. Both were cross-bred predominantly of the Romney breed. During stage A of the experiment, from 18 September to 2 October, keds on the lamb were counted daily, the periods fluctuating in length by several hours. In stage B, from 2 to 27 October, the consecutive periods were 4, 6, 4, 1 and 10 days. Keds found at each count were scattered over the sides of the ewe.

The numbers of keds found on the lamb during stage A were relatively small in comparison with the population on the ewe and therefore could have caused little disturbance when returned to it. Those found during the longer periods of accumulation of stage B represented a larger proportion of those on the ewe and accordingly their return to the latter must have caused greater disturbance to the population pattern.

Keds on the ewe were counted on 19, 23 September and 16 October, a sufficient number of dates to indicate the population trend without unduly interfering with their natural distribution in the fleece. Following each count the parasites were returned to this sheep.

The ewe and the lamb grazed in a paddock with a few other ked-infested adult sheep. As the pair were tame, they were easily caught without flocking of the inhabitants of the paddock.

III. HABITS OF THE EWE AND THE LAMB

The writer spent a few hours a day in the paddock on most days of the experiment and observed that both animals kept aloof from the others and rarely had bodily contact with them.

When feeding from its mother the lamb would swing round so that its posterior end was directed towards the anterior end of the ewe's body. Thus the regions of ewe's fleece which had most frequent and prolonged contact with the lamb were the belly immediately in front of the udder and the lower part of the posterior half of the side.

In transition from a milk diet to one in which milk was increasingly supplemented by grass the lamb behaved in much the same way as is customary. Feeding periods and attempts to feed from the mother declined in number and, with the growing independence engendered by longer grazing, accidental bodily contact also decreased. In order to obtain some idea of the rate of decline in the number of contacts with the mother, the feeding habits of two other lambs were briefly observed. One of these was 12 days and the other 7 weeks of age. Thus they approximated in age to the lamb of the present experiment, at the beginning and at the end of the experiment. Between 10.20 a.m. and 1.24 p.m. the younger lamb fed or attempted to feed from the mother ten times, the process involving a total of 175 sec. contact. In addition there were two other contacts between them. Except when lying down, the lamb kept in the close vicinity of its mother and nibbled grass occasionally. The 7-week-old lamb, between 11 a.m. and 1·13 p.m. on the same day, had three contacts with its mother, two being for feeding, which occupied 38 sec.; at one stage it grazed continuously for 38 min. Thus the opportunities for any transfer of keds to the older lamb from its mother were considerably fewer than they were for the younger lamb. It is concluded, therefore, in the present experiment, that the opportunities for transfer of keds from the ewe to the lamb diminished with the passage of time, and that by the end of the experiment it was conceivable that the daily rate of transfer would have accordingly declined.

IV. ORIGIN OF KEDS ON THE LAMB

From the observations made on the habits of the ewe and the lamb it is concluded that, so far as vectors were concerned, there was an almost closed ewe–lamb system.

Since the pupal period of the ked lasts approximately 21 days, natural increase may be discounted as of significance in contributing to the populations on the lamb.

Graham & Taylor (1941) and Evans (1950) record that many keds reach the

ground from sheep, and in the present experiment there is evidence of this. That such vagrants were a prominent factor in the present experiment seems unlikely. The few hundred which may have found their way to the ground during the 39 days of observation must have been thinly spread. Further, their life apart from sheep is short. Search for them by dragging sheep skins over the grass was unsuccessful and the conclusion is drawn that, if the lamb acquired vagrants, they could not have been accumulated sufficiently rapidly to disguise the population trend resulting from the lamb's frequent contact with its heavily infested mother.

V. VECTOR POTENTIAL OF THE EWE

The population of keds on the ewe for the dates 19, 23 September and 23 October was respectively 263, 295 and 310, and for the ewe–lamb system respectively 284, 328 and 346 (see Table 2).

For over a year before the experiment began the ewe had been used for other experiments, in the course of which numerous larvae were deposited and completed development. From the beginning of July, immediately preceding the experiment, the population on this sheep was permitted to increase without interruption. Between 25 July and 11 September, on eight dates, tallies were made of pale pupae (indicative of recent larviposition) and the numbers found ranged from 3 to 8 with an average of 5·4. The actual daily average larviposition must have been larger, because pupae darken within 24 hr. and one could not be certain that all were found. Since larvae deposited from late August onwards were due to hatch during the experimental period the above-mentioned average can be taken as an indication of the expected population increase, on which basis about 150 keds would be expected to emerge in the 28 days between the first and last counts on the ewe. This rate of increase was not revealed in the populations actually found and it is concluded that many keds disappeared from the ewe–lamb system.

The sex ratio of the keds on the ewe changed, in the course of the experiment, from 1 male to 1·04 females at the first count to 1:1·58 at the third. From pupal hatchings Graham & Taylor (1941) found that the sexes emerge in equal proportions, so that the change in the sex ratio on the ewe could not have been caused by natural increase. It follows that, among the keds disappearing from the ewe–lamb systems, males predominated.

There were features of the distribution of keds on the ewe which had a bearing on their transfer to the lamb. On 23 September the sex ratio of keds in the regions anterior to and posterior to the navel was determined. In the former there were 71 males and 137 females and in the latter the ratio was 51 to 36. Table 1 contains the number and sex ratio of keds that were found on various dates in a zone extending forward 8 in. from the udder and from flank to flank of the belly. It is seen that males predominated, an average of 5·6–1·9 females being present. In view of the frequency with which lambs come into contact with the hinder part of the ewe when feeding, the above observations indicate that there was the possibility of transfer of a greater proportion of the males than the females to the lamb.

During stage A of the experiment the numbers of keds present on the surface of the ewe's fleece were counted when tallies were made on the lamb. Six of the daily examinations occurred between 10 a.m. and 1.30 p.m. and in three of these keds were found, the total being eighty-seven; eight examinations occurred between 3.30 p.m. and 5.30 p.m., when two keds were found. Thus the dissemination potential of the ewe was much higher during the midday period.

Table 1. *The numbers of the sexes found in the pre-udder region of the ewe on various dates*

Date	Time (p.m.)	Males	Females
Sept. 22	4.00	12	4
23	4.00	3	1
28	6·00	5	1
29	12.30	0	0
30	5.30	6	5
Oct. 1	6.00	4	1
6	5.30	6	2
12	3.30	6	3
16	12.30	8	0
Average		5·6	1·9

VI. POPULATIONS ON THE LAMB AND LENGTH OF PERIOD

Throughout the experiment keds applied themselves closely to the skin of the lamb, except for a few individuals, some of which were caught in the act of transfer from the mother. The majority favoured the under parts of the body, regions not prone to come into contact with other sheep. Accordingly their dissemination potential was low.

The populations found are given in Table 2 and plotted in Fig. 1 as a per hour of daylight rate of accumulation.

In stage A of the experiment (Table 2) fluctuations in populations acquired by the lamb were not closely correlated with length of period. Since the periods of darkness were near constant, the fluctuation in length of period related to hours of daylight. From Fig. 2 it can be seen that when length of period is plotted against population, a curve fitted to the points intercepts the ordinate near the 15 hr. mark. The equivalent data for stage B (Fig. 3) of the experiment, when hours of light and darkness in each period were equal or nearly equal, have a trend that passes through the origin. It is well known that keds can transfer between sheep readily during daytime and it has been shown above that the dissemination potential on the ewe was higher between the hours 10 a.m. and 1·30 p.m. than between 3.30 and 5.30 p.m. The data in Fig. 2 are therefore interpreted as meaning that for approximately 15 hr. per day the lamb acquired few, if any, keds and that this period consisted of the hours of darkness and some hours of daylight.

From Fig. 1 and Table 2 it can be seen that there was a downward trend in the rate at which the lamb acquired keds, in the presence of an increasing population on the ewe. The influence in the lamb's feeding habits, with its accompanying decline in frequency of contact between mother and lamb, is held accountable for

Table 2. *Keds acquired by a young lamb from its mother*

Stage	Date	Time	Length period (hours)	Population on lamb: Males	Females	Total	No. per hour (daylight)	24 hr. equiv. no.	Population on ewe: Males	Females	Total	Daily hours bright sunshine	Rainfall daily
A	Sept. 18	5.30 p.m.											
	19	10.00 a.m.	16·50	14	7	21	4·66	30·48	129	134	263	10·2	0
	20	11.00 a.m.	25·0	16	21	37	2·85	35·52	—	—	—	11·2	0
	21	1·00 p.m.	26·0	26	16	42	3·00	38·88	—	—	—	2·0	0
	22	3.30 p.m.	26·5	19	7	26	1·79	23·52	—	—	—	0	0·31
	23	4.00 p.m.	24·5	21	12	33	2·64	32·40	122	173	295	0	0·02
	24	5.00 p.m.	25·0	23	24	47	3·62	45·12	—	—	—	7·3	0·02
	25	5.15 p.m.	24·25	7	14	21	1·71	20·64	—	—	—	0·2	0·65
	26	10.30 a.m.	17·25	7	3	10	1·90	13·92	—	—	—	4·5	0·10
	27	1.30 p.m.	27·0	17	18	35	2·33	30·96	—	—	—	3·4	0·15
	28	5.30 p.m.	28·0	13	20	33	2·06	28·32	—	—	—	1·0	0·02
	29	12·00 noon	18·5	11	11	22	3·38	28·56	—	—	—	1·5	0·02
	30	5.30 p.m.	29·50	18	20	38	2·23	30·96	—	—	—	4·5	0
	Oct. 1	5.30 p.m.	24·0	13	4	17	1·42	17·0	—	—	—	3·2	0·08
	2	5.00 p.m.	23·5	3	7	10	0·87	10·32	—	—	—	0	0·25
B	Oct. 6	5.00 p.m.	96·0	22	41	63	1·31	15·75	—	—	—	—	—
	12	3.00 p.m.	142·0	25	61	86	1·23	14·64	—	—	—	—	—
	16	12.00 noon	93·0	13	23	36	0·80	9·36	120	190	310	—	—
	17	12.00 noon	24.0	4	16	20	1·66	20·0	—	—	—	—	—
	27	12.00 noon	240·0	48	73	121	1·01	12·1	—	—	—	—	—

this, as discussed above. Another factor may have operated in stage B of the experiment, when the periods of accumulation varied from 1 to 10 days and the average daily rate of accumulation fell lower than in the preceding stage. In this stage the longer accumulation periods than in stage A resulted in a larger population occurring on the lamb, and there must have been a corresponding depletion of

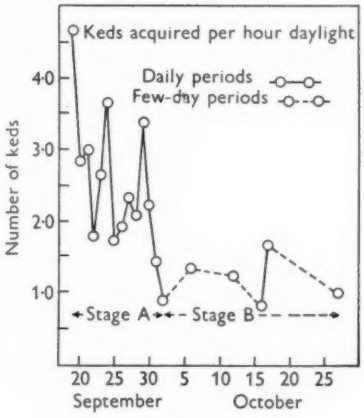


Fig. 1

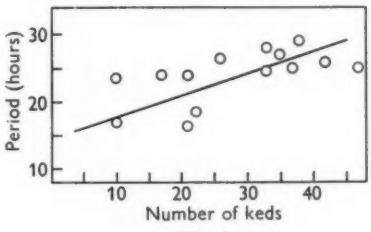


Fig. 2

Fig. 1. The rate of ked accumulation on the lamb per hour of daylight in stages A and B of the experiment.

Fig. 2. Number of keds acquired by the lamb in stage A of the experiment plotted against length of period.

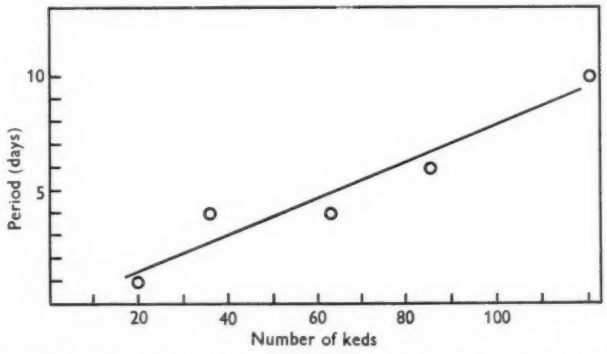


Fig. 3. Number of keds acquired by the lamb in stage B of the experiment plotted against length of period.

keds on those parts of the ewe most frequently in contact with the lamb, for this parasite travels slowly through the fleece (see Part I), and replenishment of those areas would be slow. Thus, progressively fewer keds would be expected to transfer to the lamb in the course of each few-day period. Some evidence for this is provided from the single twenty-four hour period that interrupted the stage B series

on 16–17 October, when twenty keds, more than the average daily rate for any of the other periods, were acquired by the lamb. However, in Fig. 3 it can be seen that the relation between number of keds and length of period resembled an arithmetical progression. This is consistent with there having been an almost, if not entirely, continuous traffic in keds in one direction, from the ewe to the lamb.

VII. METEOROLOGICAL FACTORS AND KED DISSEMINATION

In Fig. 4 daily numbers of keds acquired per hour of daylight by the lamb in stage A of the experiment are plotted in chronological order. The wide fluctuation indicates that a factor or factors other than hours of daylight are concerned in determining the rate of ked accumulation.

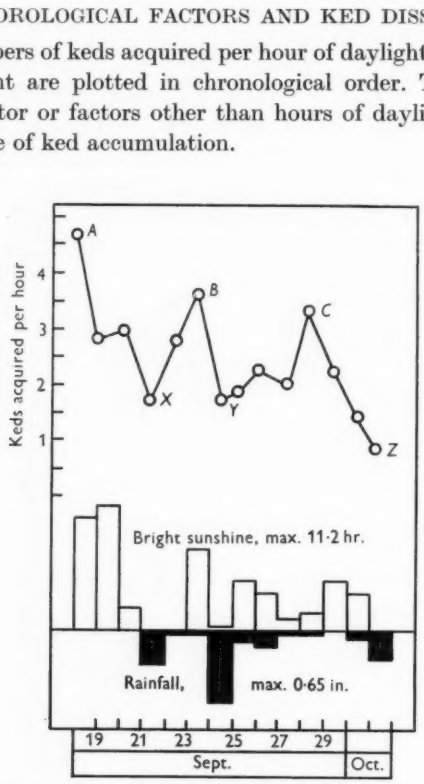


Fig. 4. The rate of ked accumulation in stage A of the experiment per hour of daylight plotted in conjunction with daily hours of bright sunshine and daily rainfall.

Factors of the microclimate of the fleece which cause keds to migrate to the surface are obviously significant in bringing about dissemination of the keds. These have been little studied. It would be expected that various factors of weather would modify the microclimate and thus indirectly affect the behaviour of the ked and its dissemination.

The meteorological data now considered suffer from the deficiency that they were collected for another purpose, that of weather forecasting. They relate to arbitrary points of time and have a periodicity differing from that of the experiment.

It is not forgotten that factors other than those pertaining to meteorology govern the behaviour of the ked. For instance, vibration caused by running of the sheep stimulates keds to appear on the fleece surface.

(i) *Humidity*

No correlation was observed between daily relative air humidity at 9 a.m. and ked accumulation on the lamb.

Daily rainfall has, to a degree, an inverse relation with the amount of bright sunshine, so that one cannot be considered to the exclusion of the other. Correlation of rainfall with ked numbers is further obscured by the fact that the degree of humidity in the fleece remains high for some time after rain has ceased. For stage A of the experiment it is shown in Fig. 4 at points *X*, *Y* and *Z* that low rates of ked accumulation were associated with low amounts of bright sunshine and heavy rainfall. Conversely at points *A*, *B* and doubtfully at *C* high rates of accumulation were connected with high amounts of sunshine and low rainfall. High rainfall and low amounts of sunshine did not prevent appreciable numbers of keds transferring to the lamb.

(ii) *Light*

It has been shown above that populations of keds acquired by the lamb were not closely correlated with the number of hours of daylight in each period, but that high rates of accumulation tended to be associated with a high number of hours of bright sunshine. In Fig. 5 the running totals of keds acquired by the lamb are

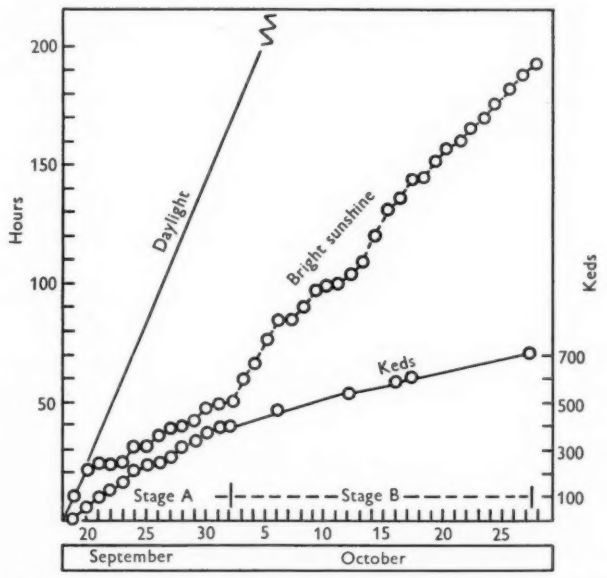


Fig. 5. Running totals of keds found on the lamb plotted in conjunction with the running totals of hours of bright sunshine and daylight. Stage A, daily counts of keds. Stage B, accumulation periods of 1 or more days.

plotted against those for hours of daylight and bright sunshine. There is a complication in comparing these, because it has previously been concluded that the growing independence of the lamb resulted in a falling away in the rate of ked accumulation. If attention be paid to stage A of the experiment, which concerned the first fortnight of the lamb's life when it was still almost completely dependent on its mother for food, it can be seen that the running totals of keds more nearly paralleled those of bright sunshine than of daylight. However, it has been shown above that keds were acquired in the absence of bright sunshine, so that the relation must have been an indirect one.

The divergence in stage B is attributed to the diminishing contact between the lamb and ewe as the former made transition from a milk to a pasture diet, with resulting less frequent opportunity for keds to transfer to the lamb.

(iii) *Temperature*

The experiment was conducted during weather in which air shade temperature maxima fluctuated between 54·6 and 65·7° F. and minima between 23·8 and 48·9°F. No connexion could be found between these data and ked populations on the lamb.

In Part I the writer concluded that keds were induced to come to the fleece surface in bright sunshine because of altered temperature relations within the fleece. In the present experiment it is consistent to believe that bright sunshine, by raising the temperature in those parts of the fleece directly exposed to the rays above a certain threshold, played a significant part in determining the dissemination potential of keds on the ewe and thus, in combination with the frequency of contact of the ewe and lamb, the rate of ked accumulation on the latter. While the factors that modified the temperature, such as intermittent rain and wind (daily anemometer reading ranging from 92 to 221), complicate correlation of daily rate of ked accumulation with fleece temperature change, as indicated by daily hours of bright sunshine, consideration of the data in terms of running totals reveals the trend.

VIII. DISSEMINATION INDEX OF KEDS

The term dissemination index is defined as the percentage of the population of the vector which daily reaches a second host. In stage A of the experiment the indices, calculated from daily averages in the data of Table 2, were for the period 18–25 September, males—14·4 %, females—9·5 %, and from 25 September to 2 October respectively, 9·6 and 6·8 %. During stage B the figures were 3·8 and 4·6 %. Thus for stage A, at least, a greater proportion of males than females transferred from the ewe to the lamb.

The data for stage A are at variance with what was found in Part I for fully fleeced sheep, where it was shown that the dissemination potentials of the sexes were equal. The divergence may be explained in terms of the ked-sheep pattern. Above it has been shown that during feeding a lamb comes into more frequent contact with the ewe in the vicinity of the udder and also that males outnumbered females in the hinder parts of the ewe. Thus the stage was set for a greater proportion of males than females to transfer from the ewe to the lamb.

It is possible that in stage B the longer accumulation periods may have led to disappearance of males from the lamb to a greater degree than females, as was observed above for the ewe. Further, the transfer to the ewe of the relatively large populations found on the various dates of this stage, may have disrupted the natural distribution on the ewe, and accordingly the proportions of the sexes available to the lamb.

IX. POPULATION PATTERN ON THE LAMB

From the frequency with which keds were found on the head of the lamb, up to six being found at an examination date, and the rarity with which keds are found on the heads of lambs after they have ceased to take milk, it was apparent that many keds gained access to the lamb in its anterior end while it was taking milk. On one occasion two males were caught in the act of transfer to the head of the lamb when it attempted to feed.

Table 3. *The population of keds found on the region of the lamb anterior to a line at the level of the navel compared with the numbers found posterior to this line. Times of examination as for Table* 2.

		Anterior region			Posterior region			Total both sexes
Stage	Date	Males	Females	Total	Males	Females	Total	
A	Sept. 21	9	11	20	17	5	22	42
	22	8	1	9	11	6	17	26
	23	16	11	27	5	1	6	33
	24	10	14	24	13	10	23	47
	25	4	8	12	3	6	9	21
	26	6	2	8	1	1	2	10
	27	3	14	17	14	4	18	35
	28	6	9	15	7	11	18	33
	29	4	4	8	7	7	14	22
	30	5	9	14	13	11	24	38
	Oct. 1	7	3	10	6	1	7	17
	2	3	6	9	0	1	1	10
	Total	81	92	173	97	64	161	334
B	Oct. 6	6	29	35	16	12	28	63
	12	6	20	26	19	41	60	86
	16	5	15	20	8	8	16	36
	17	1	9	10	3	7	10	20
	27	23	46	69	25	27	52	121
	Total	41	119	160	71	95	166	326
	Grand total	122	211	333	168	159	327	660

In Table 3 the distribution of keds for two main regions on the lamb, anterior to a vertical line at the level of the navel and posterior to that line, is given. Daily averages of 6·7 and 8·1 males were found on the respective regions during stage A of the experiment and for females 7·7 and 5·3. In stage B the daily averages were, for males—1·6 and 2·8 and for females—4·8 and 3·8, respectively. That is, associated with the longer accumulation periods of stage B of the experiment, the males

had an increased proportion of their population in the posterior region. The ratio of females in the two regions was unaffected by length of period. This is interpreted as meaning that males have a more pronounced impulse than females to move over the lamb. In Part I it was found that males would move several times the distance of females in a given period.

X. SUMMARY

The daily rate at which a young lamb acquired keds from its mother was studied. Keds applied themselves closely to the skin of the lamb, many on the under, sheltered parts of the body. It is concluded that there was almost completely one-way traffic of keds from the ewe to the lamb. A fall in the rate of transfer of keds to the lamb was attributed to declining frequency of contact between the latter and its mother, associated with transition from a wholly milk diet to one in which pasture became of increasing importance. It was found that during the hours of darkness and some hours of daylight few, if any, keds were acquired by the lamb. Keds tended to accumulate on the lamb in greater numbers during periods of many hours of bright sunshine and little rain than those of few hours of sunshine and much rain. The running totals of keds acquired by the lamb more nearly paralleled the running totals of hours of bright sunshine than other meteorological factors. It is concluded that the connexion was indirect and that sunshine raised the temperature of the fleece of the mother in the regions directly exposed to the rays, causing greater numbers of keds to appear on the surface, so that the lamb was exposed to a risk of heavier infestation. At one stage of the experiment a greater proportion of the males than females transferred from the ewe to the lamb. This is explained in terms of there being a higher proportion of males in the hinder part of the body of the ewe, a region with which the lamb, in feeding, came more frequently into contact. The higher ratio of males to females in the posterior half of the lamb than in the anterior is explained by the more pronounced habit of males than females to migrate through the fleece.

REFERENCES

EVANS, G. O. (1950). Studies on the bionomics of the sheep ked, *Melophagus ovinus* L., in west Wales. *Bull. Ent. Res.* **40**, 459–78.

GRAHAM, N. P. H. & TAYLOR, K. L. (1941). Studies on some ectoparasites of sheep and their control. I. Observations on the bionomics of sheep ked (*Melophagus ovinus* L.). *Pamphl. Coun. Sci. Industr. Res. Austr.* no. 108, pp. 7, 9–26.

TETLEY, J. H. (1958). The sheep ked, *Melophagus ovinus* L. Part I. Dissemination potential. *Parasitology* **48**, 353.

(*MS. received for publication* 25. VII. 1957.—Ed.)

A NEW SCHIZOGREGARINE, *MATTESIA ORCHOPIAE* N.SP., IN A FLEA OF SQUIRRELS IN ENGLAND

By BISWAPATI DASGUPTA

Department of Parasitology, London School of Hygiene and Tropical Medicine, London

(With 14 Figures in the Text)

INTRODUCTION

In October 1956 a batch of fleas collected from squirrel nest-boxes at Alice Holt, Surrey, was supplied by Mrs Monica Vizoso of the Infestation Control Division, Ministry of Agriculture, Fisheries and Food, for examination. It was suspected that these fleas might be responsible for carrying the *Hepatozoon* infection in the squirrels. As I was interested in the cytochemistry of various blood parasites, specially in their invertebrate hosts, I examined these fleas in serial sections for the possible presence of stages of *Hepatozoon* which could be useful for my work, and subsequently I examined more fleas collected by Miss Kamani Meedeniya from various places in Essex and Kent.

The fleas were identified by the British Museum of Natural History as *Orchopeas wickhami*, which is an ectoparasite of grey squirrels (*Sciurus carolinensis*), but is also known to infect red squirrels (*S. vulgaris*).

In the course of the preliminary examination, I came across the stages of a parasite infecting the fat-cells of this insect. Later on, some other stages of this parasite were found in the Malpighian tubules of these insects. The following is a brief description of this parasite, which is identified as a new schizogregarine of the genus *Mattesia* Naville, 1930, emend. Weiser, 1954, and for which the name *M. orchopiae* n.sp. is proposed, after the name of the host insect.

The findings regarding the *Hepatozoon* infection will follow in a separate communication.

METHODS

The abdomens of the fleas were fixed in Carnoy's fluid, dehydrated in dioxan, and cut 5–7μ thick after double imbedding in methyl benzoate-celloidin and paraffin. The sections were stained with Ehrlich's haematoxylin-eosin and with iron-haematoxylin. Some of the sections were subjected to Feulgen's nucleal reaction after 10 min. of hydrolysis in normal hydrochloric acid at 60° C. Some sections were stained with Unna–Pappenheim stain and also with toluidene blue, both before and after digestion with ribonuclease, and appropriate controls were incubated in distilled water. Several sections were stained with alcian blue.

The drawings were made with the help of a *camera lucida*.

OBSERVATIONS

The parasite at different stages of its life-history infected the fat-cells and the Malpighian tubules. The schizogony was confined to the fat-cells. The gametogony and the sporogony occurred in the Malpighian tubules.

Schizogony

Micronuclear schizonts

This stage occurred in the fat-cells, but was rather rare. The size averaged $13{\cdot}7 \times 11\mu$. They were usually globular in shape (Fig. 1), and sometimes had small irregular lobes (Fig. 2). The nuclei appeared as small deeply stained dots. With the toluidine-blue stain the cytoplasm appeared pale violet, while the nuclei stained orthochromatically.

Macronuclear schizonts

A large number of the macronuclear schizonts were encountered in the fat-cells. Their size before the formation of merozoites averaged $37{\cdot}2 \times 28{\cdot}9\mu$. These forms were globular (Fig. 3) or elongated (Figs. 4, 5) in shape with a constriction in the middle (Fig. 5), and sometimes had small lobes. The nuclei stained well with haematoxylin, but only faintly with Feulgen's technique.

After staining with toluidine blue, a large number of metachromatic granules were found in the cytoplasm of these schizonts. In sections digested with ribonuclease most of these granules disappeared. Metachromasia was not affected in the sections incubated in distilled water. A few granules staining with alcian blue occurred in the cytoplasm of these schizonts.

Merozoites of the second schizogony

The merozoites arose as finger-like projections from the very large parasitic body (Figs. 6, 7), which at this stage measured $55{\cdot}1 \times 41{\cdot}3\mu$. The merozoites measured $12{\cdot}3$–$14{\cdot}1 \times 1{\cdot}7\mu$. The nucleus of the merozoite stained well with haematoxylin, but was Feulgen-negative. Close examination, however, revealed the

---

Fig. 1. A micronuclear schizont lying in the fat-cell. (Toluidene blue.) The nuclei of the parasite and the cytoplasm of the host cell take up deep orthochromatic stain.

Fig. 2. A micronuclear schizont in the host cell. (Iron haematoxylin.) The two elongated black streaks in the host cell represent nuclei.

Fig. 3. A macronuclear schizont in the host cell. (Ehrlich's haematoxylin.)

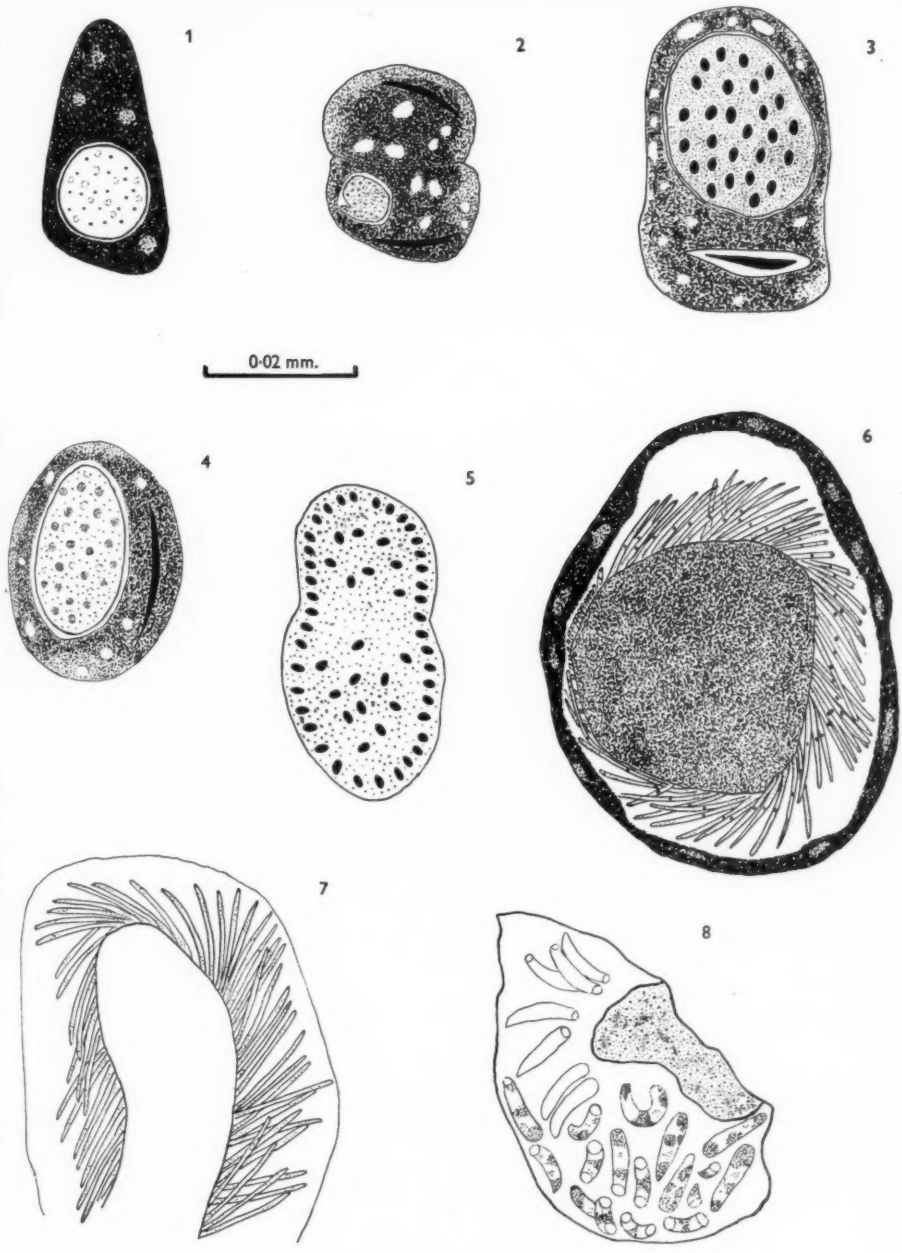
Fig. 4. A macronuclear schizont in the host cell. (Iron haematoxylin.)

Fig. 5. A macronuclear schizont. (Ehrlich's haematoxylin.) The host cell is not shown.

Fig. 6. Merozoite formation. (Toluidene blue.) The nuclei of the parasite and the cytoplasm of the host cell stains orthochromatically. Metachromatic granules are shown as minute dots in the cytoplasm of the merozoites.

Fig. 7. Merozoite formation. (Feulgen's nucleal reaction.) Minute Feulgen-positive granules are shown as dots in the cytoplasm of the merozoites. The nuclei are Feulgen-negative.

Fig. 8. Trophozoites lying near the residual mass. Those at the lower side of the picture are meant to show the basophilic areas in the body as seen with pyronin stain. Those at the upper side of the picture illustrate the Feulgen-negative reaction. The residual mass contains basophilic granules.



For legends see foot of opposite page.

presence of extremely minute Feulgen-positive particles, which appeared to be scattered in the cytoplasm of these merozoites.

Metachromatic granules, similar to those observed in the cytoplasm of the macronuclear schizonts, were found in the cytoplasm of the merozoites after staining with toluidine blue. The residual body representing the central mass of the parasite at this stage took up strong orthochromatic stain.

Trophozoites of the second schizogony

The trophozoites which measured $14{\cdot}2 \times 3{\cdot}5\mu$ were found lying near the residual mass (Fig. 8) and were also found wandering in the haemocoele in the vicinity of the Malpighian tubules (Fig. 9). Some of them also lay in the epithelial wall of the tubule (Fig. 9). The nucleus of the trophozoite was Feulgen-negative.

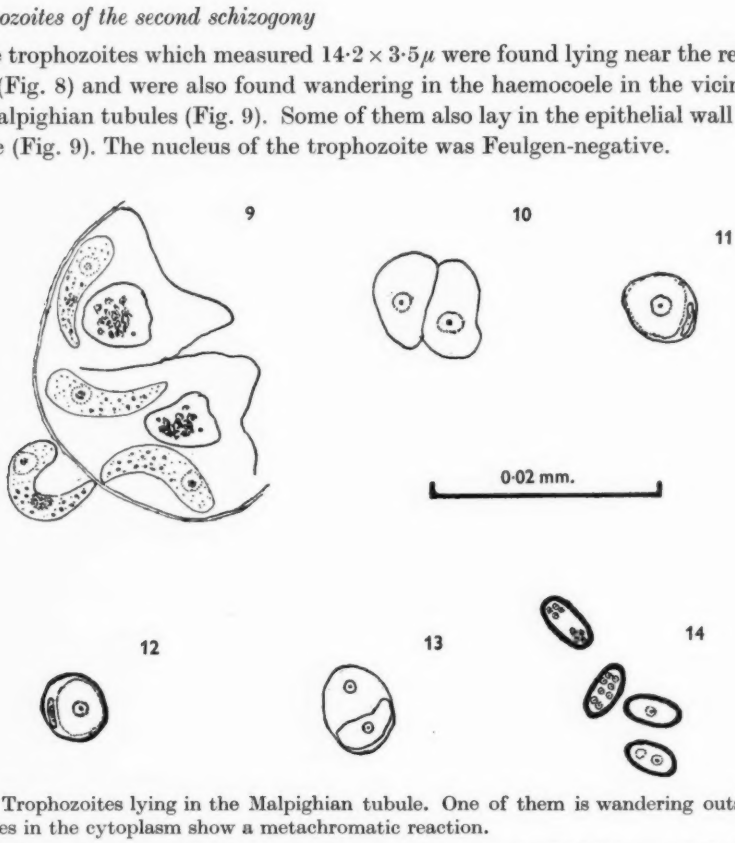


Fig. 9. Trophozoites lying in the Malpighian tubule. One of them is wandering outside it. Granules in the cytoplasm show a metachromatic reaction.

Fig. 10. Two gametocytes forming a gametocyst as seen in the Malpighian tubule. Weak Feulgen's reaction.

Figs. 11–13. Single sporoblast invested by a protoplasmic sheath containing a nucleus. Weak Feulgen's reaction.

Fig. 14. Spores invested by a thick wall, as seen in sections stained with Ehrlich's haematoxylin.

With toluidine-blue stain several metachromatic granules were seen in the cytoplasm of the trophozoites. These granules were similar to those observed in the merozoites and in the second schizonts. With Unna–Pappenheim stain, red-staining (pyronin) areas were noticed in the trophozoites (Fig. 8).

Gametogony and sporogony

It appeared that the trophozoites transformed themselves into gametocytes, and that two of them associated to produce the gametocyst (Fig. 10). These stages were extremely rare in my preparations. The nuclei at this stage were faintly Feulgen-positive.

The next stage found in the Malpighian tubule was the advanced gametocyst with a single sporoblast, which was invested by a small nucleus and a thin layer of cytoplasm (Figs. 11–13). The nuclei at this stage were faintly Feulgen-positive and the cytoplasm was intensely basophilic.

The spore, which was invested by a thick wall (Fig. 14), developed from the sporoblast and lay embedded in the cells of the Malpighian tubule, or it projected in the lumen of the same. In sections these thick-walled spores measured $10{\cdot}5 \times 7\mu$. A maximum of eight nuclei could be detected in these spores.

Feulgen's reaction could not be determined in the nuclei of the spores. The protoplasm inside the spore was strongly basophilic and stained orthochromatically with toluidene blue.

DISCUSSION

Weiser (1954*b*, 1955*a*, *b*) reviewed the classification of Schizogregarinida proposed by various workers from time to time, and formulated a new system of classification based on the developmental cycle of these parasites. This new classification is followed in this study.

According to Weiser's classification the order Schizogregarinida comprises two different families, the Ophryocystidae and the Caulleryellidae. The members of the former undergo two schizogonies, while those of the latter undergo one schizogony only. The parasite described here clearly belongs to the family Ophryocystidae.

The family Ophryocystidae is further subdivided into two subfamilies, the Ophryocystinae and the Machadoellinae. They differ from each other in that the second schizont of the former is morphologically distinguishable from the trophozoites, while in the latter the shape of the second schizont corresponds to that of the trophozoites. The parasite described here accordingly belongs to the subfamily Ophryocystinae.

The subfamily Ophryocystinae includes a number of genera distinguishable mainly by the number of spores produced in the gametocyst. On this criterion the present parasite clearly belongs to the genus *Mattesia* established by Naville (1930) and later amended by Weiser (1954*a*). This genus is usually characterized by two spores (disporic forms), but in some cases by one spore (monosporic form) only. Unequal development and retardation of one-half of the cyst may result in the formation of these two types of cysts (Weiser, 1954*a*, *c*).

Two species of the genus *Mattesia* have been previously described, *M. dispora* (Naville, 1930), and *M. povolnyi* (Weiser, 1952). The former is parasitic in the fat-cells of *Ephestia kühniella* and *Plodia interpunctella*, while the latter is parasitic in the Malpighian tubules of *Homeosoma nebulellum*. It is interesting to notice that

the parasite described here spends part of its life-cycle (schizogony) in the fat-cells, while the rest of the life-cycle (gametogony and sporogony) is spent in the Malpighian tubules of the host. Hence, as regards the site of the infection in the host, the parasite described here is intermediate between the other two species of genus *Mattesia*.

A comparison of the parasite here described with *M. dispora* and *M. povolnyi* as regards the details of their life-history leaves no doubt that it is necessary to erect a new species to receive this parasite. Accordingly, this parasite is named *M. orchopiae*, n.sp., according to the generic name of the host insect *Orchopeas wickhami*.

	Mattesia dispora	*Mattesia povolnyi*	*Mattesia orchopiae*
Host	*Ephestia kühniella* and *Plodia interpunctella*	*Homeosoma nebulellum*	*Orchopeas wickhami*
Site of infection	Fat-cell	Malpighian tubule	Fat-cell and Malpighian tubule
Situation	Intracellular	Epicellular	Intracellular
1st schizont	$8–9 \times 2–2{\cdot}5\mu$	$30–60 \times 1–4\mu$	$13{\cdot}7 \times 11\mu$
Spore	$14 \times 7{\cdot}5\mu$	$11 \times 5{\cdot}6\mu$	$10{\cdot}5 \times 7\mu$

The possibility is not excluded that there may exist two types of *Mattesia orchopiae*, the monosporic form and the disporic form, as has been reported in the case of *M. dispora* (Weiser, 1954*a*, *c*), though monosporic forms only were noticed in the specimens studied.

An interesting point of difference between the micronuclear and the macronuclear schizonts is the presence of metachromatic granules in the latter and their absence from the former. The nature of these metachromatic granules can only be conjectured. As they could be to some extent removed by digestion with ribonuclease, it appeared probable that they contained pentose nucleic acid. The presence of granules which stained with alcian blue was probably an indication of the presence of mucoid substances (Steedman, 1950). The latter conclusion appears to be supported by the fact that metachromasia could not be entirely removed by treatment with ribonuclease.

SUMMARY

A new schizogregarine *Mattesia orchopiae*, n.sp., parasitic in the flea *Orchopeas wickhami*, is described. The schizogony takes place in the fat-cells, while the gametogony and the sporogony occur in the Malpighian tubules. The micronuclear schizonts measured $13{\cdot}7 \times 11\mu$. The trophozoites of the second schizogony measured $14{\cdot}2 \times 3{\cdot}5\mu$. The spores measured $10{\cdot}5 \times 7\mu$.

After staining with toluidene blue metachromatic granules were found in the cytoplasm of the macronuclear schizonts and in the merozoites and the trophozoites resulting therefrom. Some of this metachromatic material could be removed by digestion with ribonuclease, while the rest of it persisted. Material stained with alcian blue was detected in the cytoplasm of the macronuclear schizonts.

I wish to thank Professor P. C. C. Garnham for encouragement and advice received during the conduct of this investigation, Mrs M. Vizoso and Miss K.

Meedeniya for the supply of the fleas, the authorities of the British Museum for the identification of the fleas, and Sir W. P. MacArthur for his advice regarding the problem of nomenclature.

REFERENCES

NAVILLE, A. (1930). Recherches cytologiques sur les schizogrégarines, I. Le cycle évolutif de *Mattesia dispora* n.g., n.sp. *Z. Zellforsch.* **11**, 375–96.

STEEDMAN, H. F. (1950). Alcian blue 8 GS: a new stain for mucin. *Quart. J. Micr. Sci.* **91**, 477–9.

WEISER, J. (1952). Cizopasníci housenek zaviječe slunečnicového *Homeosoma nebulellum* Hbn. se zvláštním zřetelem na druh *Mattesia povolnyi* sp.n. *Zool. ent. listy*, **1**, 252–64.

WEISER, J. (1954*a*). Schizogregariny z hymzu škodícího zásobám mouky II. *Mattesia dispora* Naville 1930 a *Coelogregarina ephestiae* Ghelelovitch 1947. *Mém Soc. zool. tchécosl.* **18**, 73–90.

WEISER, J. (1954*b*). Příspěvek k systematice schizogregarin. *Čsl. Parasitology*, **1**, 179–212.

WEISER, J. (1954*c*). Zur systematischen Stellung der Schizogregarinen der Mehlmotte, *Ephestia kühniella* Z. *Arch. Protistenk.* **100**, 127–42.

WEISER, J. (1955*a*). A new classification of Schizogregarina. *J. Protozool.* **2**, 6–12.

WEISER, J. (1955*b*). A new classification of Schizogregarina. A correction. *J. Protozool.* **2**, 88.

(*MS. received for publication* 25. x. 1957.—Ed.)

THE BIRD FLEAS OF FAIR ISLE

By MIRIAM ROTHSCHILD

(With 10 Figures in the Text)

INTRODUCTION

During 1951–56 Williamson and his collaborators collected ectoparasites from the bodies of birds caught on Fair Isle. The fleas (except for those caught in 1956) were sent to me for determination. This collection has proved most valuable and interesting, principally because Williamson (1954) has evolved a brilliant 'de-fleaing' technique which has revolutionized collecting from living hosts. The fact, however, that I could not visit Fair Isle has proved a serious drawback in many ways. From a distance the data have proved difficult to handle, and despite a voluminous correspondence—since both parties concerned are tenacious letter-writers!—much of the information obtained in one season is not strictly comparable with that obtained for the subsequent and previous seasons. Thus, for example, the negative records for 1954 were given daily or monthly, but in 1955 were undated and lumped together under 'autumn' or 'spring' migrants, or 'summer broods'. In 1953 no negative records were available. In the case of some hosts, such as the robin, the subspecies were distinguished in some seasons but lumped under the name 'Robin' in others. In 1951, owing to a shortage of tubes, a number of fleas from the same species of host were put in one container and it afterwards proved impossible to sort species or sex according to dates. In addition, there were sometimes discrepancies between the lists of the material dispatched and the material actually received. Thus, from time to time, the list recorded 'three fleas', whereas the tube was found to contain four or vice versa. I was also responsible for losing some specimens, including the tube containing the 'record' number of fleas from one bird—twenty-two specimens from a juvenile starling. There were also a number of discrepancies in lists of negative resident birds, all of which were compiled from the laboratory slips supplied by different assistants at the ringing station. This was most probably due to the manner of scoring retraps which apparently varied with the individual or sometimes with the season! The worst example of this sort involved the resident wheatears in 1951 when there proved to be a difference of twenty-one negative records (out of a total of 117) between the two lists. This discrepancy affected the calculated infestation rate in this particular batch by 0·4 %. Williamson and I decided the labour involved in rechecking these lists was out of all proportion to the advantages we should obtain by the corrections in question. In view of these disappointing inaccuracies—of which I am all too conscious—it is most important not to lay stress on detail in the following account but only to emphasize the broad general trends which have emerged from the study of this collection.

It must also be noted that the various paragraphs relating to fleas published in the *Fair Isle Bird Observatory Bulletin* were written by Kenneth Williamson and were

based on my notes and letters commenting upon the specimens as I received them piecemeal. Certain corrections have been made as a result of rechecking the material and comparing the collection as a whole.

It should be noted that I have amalgamated the various host subspecies under their specific names except in the case of the wheatears, since one of its subspecies is a breeding summer resident on Fair Isle and the other two passage migrants. These are considered separately.

METHODS

The majority of the birds were trapped by means of the five permanent Heligoland traps of the ringing station which extend for about a mile south of the Observatory itself. The birds were 'de-fleaed' by passing them through chloroform fumes in the Fair Isle chloroform-vapour apparatus by the method described by Williamson (1954). This procedure was shown to be 100% efficient in removing parasitic flat flies (Corbet 1956, p. 405), but Williamson (p. 234) considers it only slightly less effective for fleas in the case of small bird hosts. No systematic tests were carried out to prove this point, but it seems likely that occasionally anaesthetized fleas would get caught up in the plumage and thus escape notice.

FAIR ISLE AND THE BIRD HOSTS

Fair Isle is situated 24 miles south-south-west from the southernmost point of the Shetlands and 26 miles east-north-east of the most northerly point of the Orkneys and is thus approximately 25 miles from the nearest land. It is oblong in form, having an extreme length of about $2\frac{3}{4}$ miles from north to south and averaging about 1 mile in breadth—that is approximately 3 square miles in area. There are no trees or shrubs (apart from a few bushes in gardens beside the crofts) on the island which is surrounded by precipitous cliffs 100–600 ft. high except in the extreme south-west. The northern two-thirds are chiefly heathland and rough moorland pasture, and it is here the main trapping in the five permanent Heligoland traps is carried out. Small areas of richer pasture land and patches of cultivated arable land are situated in the southern end of the island. Fair Isle lies in the path of much European drift migration (Williamson, 1952, 1953) and its isolated position turns it into a veritable 'bottle-neck' on autumn and spring passages for both continental and Iceland/Greenland birds.

The great majority of the migrants from which fleas were obtained were Passerines. The non-Passerine migrants are larger and more difficult to handle or pass through the Fair Isle apparatus, and Williamson believed that the infestation rate of those birds which were examined was possibly higher than we recorded. None of the larger cliff-dwelling resident birds of Fair Isle were 'de-fleaed'. Apart from the migrants, fleas were obtained from eight breeding Passerine birds, the wheatear (*Oenanthe oenanthe*), the rock pipit (*Anthus spinoletta*), the meadow pipit (*Anthus pratensis*), the twite (*Carduelis flavirostris*), the starling (*Sturnus vulgaris*), the house sparrow (*Passer domesticus*), one brood of nestlings of the wren (*Troglodytes*

troglodytes), and the blackbird (*Turdus merula*) of which only one pair nested on the island.

It is convenient to add the crossbill (*Loxia curvirostra*) to this group, of which we examined 120 in 1953, although not a breeding bird on Fair Isle, this is a summer 'irruption' species, many individuals remaining on the Island for some time, and so far as exposure to infestation is concerned, can rank as a summer resident.

Among the breeding birds the wheatear, meadow pipit, and the majority of the twite are summer residents—arriving in the spring and departing again in the late summer or autumn. The sparrows, starlings, rock pipits, at least some of the twites and skylarks (*Alauda arvensis*), the few scattered pairs of wrens and the solitary pair of blackbirds are residents throughout the year. However, there are also migrant blackbirds and starlings passing through the island which add to their numbers at certain seasons. From the middle of August onwards the southward stream of wheatear migrants begins to pass through the island and these are mostly indistinguishable from the residents—a fact one greatly deplores as it makes a comparison of the infestation rate of spring and autumn migrant wheatears impossible.

During the period under review about 5000 birds (78 species) were examined for fleas (we have records of 4913 of these) of which all but 59 (19 species) were Passerine birds. The resident breeding birds, wheatears, starlings, rock pipits, meadow pipits and twites accounted for about three-quarters of this total.

The wheatear population on Fair Isle is considerable. Butterfield (1952) estimated the number of breeding pairs to be 800. In a previous paper (Rothschild, 1955) I drew attention to the fact that Condor states that on Skokholm Island the wheatear does not return to old nesting sites. J. F. Thompson confirmed this observation for the pairs he watched for me at Laugharne Burrows, Carmarthenshire and other Welsh localities. It is not, however, the case for the wheatears on Fair Isle. Both Williamson (personal communication) and Corbet (1956) are emphatic on this point. Frequently, on Fair Isle the wheatear does return to old nesting sites. This may also apply to the rock pipit, a bird which nests in holes and crevices mainly on the cliffs and never far from them. The meadow pipit (which was not recorded as a breeding species by Eagle Clarke in 1912) nests on the open ground among grass and heather some distance from the cliffs, in a similar environment to the twite. Neither of these birds, according to Corbet, uses the same nest or nesting site in consecutive years. The starlings which nest almost entirely in holes in the field walls return most consistently, here as elsewhere, to their old nesting sites. Very few sparrows were examined (22, one infested), and only 3 skylarks, all of which were negative, One or two skylarks' nests also proved to be negative.

The wheatear is single-brooded on Fair Isle and late young birds are thought to be due to a failure to rear a brood at the first attempt. The meadow pipit, rock pipit, twite and the starling are all double-brooded. Williamson informs me that the pipits build a new nest for their second brood, whereas those starlings which breed again use the same nest (only about 20% appear to raise a second brood). The nesting dates do not vary much from year to year. Both pipits breed considerably in advance of the wheatear and are feeding first-brood young in the last week of

May and first week of June, whereas the former species commences feeding young in the nest during the last fortnight of June and the first week of July.

One season (personal communication from Williamson) starlings commenced breeding a week late and then reared only a single brood.

After leaving the nest the juvenile starlings form flocks which may number up to a hundred birds or more. Fair Isle is small and limited with regard to choice of habitat. The flocks of juvenile starlings feed chiefly on moorland—largely over the same terrain as the wheatears and pipits. Most of the small ground-feeding Passerines are in fairly close association for much of the late summer. These juvenile birds tend to follow each other into the traps which they enter and re-enter. Adult resident birds are rarely caught in this fashion.

The habits of the host, such as re-nesting in old sites, the rearing of a second brood, flocking, roosting, resting and feeding behaviour must affect the infestation rate of the various species or age groups of birds concerned. It may even be that the tendency to re-enter the Heligoland traps increases the infestation rate of the young birds in question.

THE FLEA FAUNA OF FAIR ISLE BIRDS

Six species of flea were found on the birds from the traps. Of these *C. fringillae* (one specimen from a whitethroat (*Sylvia communis*)) and *C. garei* (four specimens, one each from a migrant white wagtail (*Motacilla alba*), robin (*Erithacus rubecula*), woodcock (*Scolopax rusticola*) and meadow pipit) are in all probability passage migrants with their hosts and do not breed in nests on Fair Isle. The status of *C. garei* has been fully discussed previously (Rothschild, 1955; Rothschild in Smit, 1955). It was a surprise that there was only one record of *C. vagabundus* (from a migrant turnstone) as it seems almost certain that this species breeds in nests of gulls and other suitable hosts on the precipitous cliffs round the shores of Fair Isle. One would have expected new arrivals to pick up stragglers of this species quite frequently. The distribution of *C. vagabundus* on Fair Isle is a point well worth further investigation. The three important species found were *D. g. gallinulae* (430 records or 8·4 % of all birds examined), *C. gallinae* (205 records or 3·8 % of all birds examined) and *C. borealis* (126 records or 2·3 % of all birds examined), all of which were also discovered breeding in nests on Fair Isle.

If the records for the year 1953, for which no negative data are available, are again disregarded, we find an over-all infestation rate of 13 %. Such a calculation has little value since there are such widely divergent infestation rates according to the different seasons, different species and different age groups of birds. As we shall see, the spring migrants have an infestation rate of 52·2 % compared with 2·7 % in the autumn. The migratory redstart (*Phoenicurus phoenicurus*) has an infestation rate of 75·8 % in the spring, but the swallow (*Hirundo rustica*) only 16 %. The resident starling has an over-all rate of 17·1 % and the twite only 3·8 %. Furthermore, the starling rate rises sharply by 9 % in August when the second brood young are taken in the traps. Each species of host and each species of flea would have to be considered separately in order to obtain some idea of the true infestation rate.

THE FLEA INFESTATIONS OF THE MIGRANTS

If we omit the summer and permanent residents from the list, thirty-five species of migrant birds (Table 1) were found to harbour fleas. The 1953 infestations, a year in which no negative records were available, are included but are omitted from the percentage calculations. Since only one pair of blackbirds* was known to have nested on the Island, this species is included in Table 1. The crossbill is excluded for the reason given on p. 384. These are somewhat arbitrary distinctions and it is of course realized that the summer residents are strictly speaking also migrants in the spring and autumn, but it is thought advisable to consider them with the permanent resident and breeding birds.

Out of the 379 spring migrants examined, 199 or 52·2 % (when the lack of 1953 negatives are allowed for in both seasons) were found to harbour fleas. More autumn migrants (615) were searched and of these 19 or only 2·7 % were infested. It was unfortunate that a number of spring migrants were not represented in the autumn collections, for example, the Faroe and Greenland wheatears (*Oenanthe o. schioleri* and *O. o. leucorrhoa*), could not, at this season, be distinguished from summer resident birds, thus making an accurate comparison between the two migrations impossible. The relatively high spring infestation rate, however, is clearly demonstrated in those species of host which are well represented among both northward and southward streams (see Table 5). Thus, 9 garden warblers (*Sylvia borin*) out of 17 examined were infested on the spring (northward) passage and 0 out of 32 on the autumn (southward) passage. The willow warbler (*Phylloscopus trochilus*) showed 24 out of 43 infested in the spring and only 1 out of 68 in the autumn. Thirty robins (*Erithacus rubecula*) out of 58 were positive in the spring, and only 1 out of 13 in the autumn. The common redstart (*Phoenicurus phoenicurus*) had 22 infested out of 29 examined in May, and 1 out of 8 between August and October. Furthermore, a most remarkably high infestation rate, 55·0 %, of spring migrants retrapped on the Island was recorded (Table 6), and it is exceedingly disappointing that no autumn migrant retraps are available for comparison. The percentage of infestation of summer residents (which were chiefly juveniles) retrapped on the Island was 18·6 %, and for the months August–October when the autumn migrants were passing through, only 11 %. The five triple infestations, i.e. birds infested with three species of fleas, all occurred on spring trappings. Furthermore, the number of multiple infestations, i.e. with more than one specimen, is greater for the spring migrants with a mean of 1·8 per infested bird compared with 1·4 for all other trappings (in Table 14 1953 records are omitted). Apart from the four specimens of *C. garei* (see also Rothschild, 1955, p. 300), and the single examples of *C. fringillae* and *C. vagabundus* the migrants harboured *D. g. gallinulae*, *C. gallinae* or *C. borealis*. The proportion of these three species present on the bodies of the birds was not, however, the same in the spring and autumn.

* I examined a nest of this pair after the young had flown. Several specimens of *D. g. gallinulae* emerged and one extremely aberrant *C. borealis* female which suggested an intermediate between this species and *C. columbae*.

Table 1. *Infestation of passage migrants (all years)*

	Spring passage		Autumn passage	
	Examined	Infested	Examined	Infested

Table 1. *Infestation of passage migrants (all years)*

Host	Spring passage											Autumn passage										
				D. g. gallinulae		*C. gallinae*		*C. borealis*		Other species					*D. g. gallinulae*		*C. gallinae*		*C. borealis*		Other species	
	Pos.	Neg.	No. fleas	♂	♀	♂	♀	♂	♀	♂	♀	Pos.	Neg.	No. fleas	♂	♀	♂	♀	♂	♀	♂	♀
Redwing (*Turdus musicus*)	2	—	2	1	—	—	—	1	—	—	—	3	99	5	—	—	1	4	—	—	—	—
Blackbird (*T. merula*)	12	9	19	10	3	1	—	1	4	—	—	6	153	7	—	2	1	3	1	—	—	—
•Greenland Wheatear (*Oenanthe o. leucorrhoa*)	11	5	22	5	5	1	3	5	3	—	—	—	—	—	—	—	—	—	—	—	—	—
•Faroe Wheatear (*O. o. schioleri*)	4	2	5	1	—	—	—	1	3	—	—	—	—	—	—	—	—	—	—	—	—	—
Whinchat (*Saxicola rubetra*)	2	1	4	—	2	—	—	1	1	—	—	1	10	1	—	1	—	—	—	—	—	—
Redstart (*Phoenicurus phoenicurus*)	22	7	43	10	18	1	2	5	7	—	—	1	7	1	—	—	—	1	—	—	—	—
Bluethroat (*Cyanosylvia svecica*)	2	2	6	3	2	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Robin (*Erithacus rubecula*)	30	28	46	16	15	5	2	3	4	—	1	1	12	1	—	—	1	—	—	—	—	—
Grasshopper warbler (*Locustella naevia*)	1	2	5	4	1	—	—	—	—	—	—	—	1	Nil	—	—	—	—	—	—	—	—
Sedge warbler (*Acrocephalus schoenobaenus*)	17	8	27	20	5	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Icterine warbler (*Hippolais icterina*)	1	1	2	1	1	—	—	—	—	—	—	1	25	1	—	1	—	—	—	—	—	—
Black cap (*Sylvia atricapilla*)	3	2	6	3	3	—	—	—	—	—	—	—	32	Nil	—	—	—	—	—	—	—	—
Garden warbler (*S. borin*)	9	8	23	12	9	—	2	—	—	—	—	—	2	Nil	—	—	—	—	—	—	—	—
Whitethroat (*S. communis*)	24	33	48	21	13	2	9	1	1	1	—	—	5	Nil	—	—	—	—	—	—	—	—
Lesser whitethroat (*S. curruca*)	5	7	10	—	6	—	4	—	—	—	—	1	67	1	—	1	—	—	—	—	—	—
Willow warbler (*Phylloscopus trochilus*)	24	19	47	11	23	2	8	1	2	—	—	—	2	Nil	—	—	—	—	—	—	—	—
Spotted flycatcher (*Muscicapa striata*)	4	13	6	—	6	—	—	—	—	—	—	—	15	Nil	—	—	—	—	—	—	—	—
Pied flycatcher (*M. hypoleuca*)	2	4	3	1	1	—	—	1	—	—	—	—	1	Nil	—	—	—	—	—	—	—	—
Hedge sparrow (*Prunella modularis*)	4	2	8	2	2	1	3	—	—	—	—	—	4	Nil	—	—	—	—	—	—	—	—
Tree pipit (*Anthus trivialis*)	4	2	11	4	6	—	1	—	—	—	—	—	15	Nil	—	—	—	—	—	—	—	—
White wagtail (*Motacilla alba*)	3	3	5	1	1	—	—	1	1	—	1	—	—	—	—	—	—	—	—	—	—	—
Grey wagtail (*M. cinerea*)	1	—	4	—	3	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Red-backed shrike (*Lanius collurio*)	1	1	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Linnet (*Carduelis cannabina*)	1	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chaffinch (*Fringilla coelebs*)	—	—	—	—	—	—	—	—	—	—	—	1	9	1	—	1	—	—	—	—	—	—
Reed bunting (*Emberiza schoeniclus*)	2	3	3	2	—	—	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Snow bunting (*Plectrophenax nivalis*)	—	2	—	—	—	—	—	—	—	—	—	1	5	1	—	—	—	1	—	—	—	—
Swallow (*Hirundo rustica*)	1	4	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Wryneck (*Jynx torquilla*)	1	1	11	10	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cuckoo (*Cuculus canorus*)	1	3	3	—	1	—	—	1	1	—	—	—	1	—	—	—	—	—	—	—	—	—
Long-eared owl (*Asio otis*)	1	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sparrow hawk (*Accipiter nisus*)	1	—	1	—	—	1	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—
Merlin (*Falco columbarius*)	—	—	—	—	—	—	—	—	—	—	—	2	4	3	—	—	1	2	—	—	—	—
Turnstone (*Arenaria interpres*)	—	—	—	—	—	—	—	—	—	—	—	1	1	1	—	—	—	—	—	—	1	—
Woodcock (*Scolopax rusticola*)	1	—	1	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
Common sandpiper (*Tringa hypoleucos*)	2	—	2	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Totals	199	172	377	139	128	14	38	25	29	2	2	19	475	23	—	6	4	11	1	—	1	—

Species of passage migrants found negative for fleas: Song thrush (*Turdus ericetorum*) 20 autumn; fieldfare (*T. pilaris*) 1 spring, 5 autumn; ring ousel (*T. torquatus*) 1 autumn; black redstart (*Phoenicurus ochrurus*) 1 spring, 2 autumn; melodious warbler (*Hippolais polyglotta*) 1 autumn; greenish warbler (*Phylloscopus trochiloides*) 3 autumn; reed warbler (*Acrocephalus scirpaceus*) 2 autumn; barred warbler (*Sylvia nisoria*) 12 autumn; chiffchaff (*Phylloscopus collybita*) 1 autumn; wood warbler (*P. sibilatrix*) 1 autumn; thick-billed warbler (*Acrocephalus aëdon*) 1 autumn; redbreasted flycatcher (*Muscicapa parva*) 1 autumn; great grey shrike (*Lanius excubitor*) 3 autumn; lesser grey shrike (*L. minor*) 1 autumn; goldfinch (*Carduelis carduelis*) 1 spring; siskin (*C. spinus*) 1 spring; redpoll (*C. flammea*) 22 autumn; mealy redpoll (*C. f. flammea*) 1 autumn; bullfinch (*Pyrrhula pyrrhula*) 1 autumn; brambling (*Fringilla montifringilla*) 1 spring, 14 autumn; red-footed falcon (*Falco vespertinus*) 1 spring; kestrel (*F. tinnunculus*) 1 autumn; water rail (*Rallus aquaticus*) 2 autumn; corncrake (*Crex crex*) 1 spring; ringed plover (*Charadrius hiaticula*) 9 autumn; snipe (*Capella gallinago*) 1 spring, 1 autumn; jack snipe (*Lymnocryptes minimus*) 1 autumn; green sandpiper (*Tringa ochropus*) 2 autumn; dunlin (*Calidris alpina*) 6 autumn; sanderling (*Crocethia alba*) 1 autumn; knot (*Calidris canutus*) 2 autumn; redshank (*Tringa totanus*) 3 autumn.
Total: 8 spring-passage migrants; 121 autumn-passage migrants.

• See p. 383.

In the spring 66 % of this flea population consisted of *D. g. gallinulae* and only 16 % of *C. gallinae* (for the migrants considered separately see Fig. 1). On autumn trappings, however, 70 % of the fleas were *C. gallinae* and only 25 % *D. g. gallinulae*. Furthermore, the only species of flea recorded from the retraps in May and June is *D. g. gallinulae* (Table 6).

Many of the spring migrants also harboured *C. borealis* (Rothschild, 1955, p. 298), and it seems probable—despite its absence among the retraps—that hosts such as the various warblers, the robin and flycatchers, acquired this species either on Fair Isle itself after arrival or in a similar 'bottle-neck' such as the Island of Ushant (Rothschild, 1948, p. 88), which they may pass through on their northward passage.

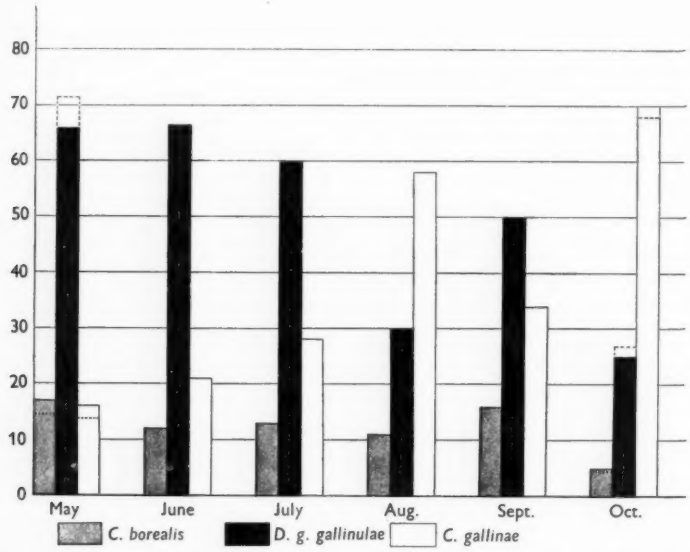


Fig. 1. Dominance. The proportion of the three common flea species in the total infestations. (The dotted line shows the proportions if autumn and spring migrants are considered separately. The two April records from the blackbird are omitted from all the figures.)

If the infestation rate of all birds trapped in May (Table 3 and Fig. 1) is considered, that is to say, recently arrived summer residents such as the wheatear and meadow pipit as well as birds on passage and permanent residents like the rock pipit, it will be appreciated that the percentage of *C. borealis* in the flea population reaches a peak in May. During this month 17 % of all fleas are *C. borealis* compared with 5–16 % in other months. The lower infestation rate with *C. gallinae* in the spring (Fig. 1) is even more striking. In the collection as a whole *D. g. gallinulae* outnumbers *C. gallinae* by 2:1 and *C. borealis* by 4:1. *C. gallinae* and *C. borealis* appear in the ratio 2:1. In the May records, however, *C. gallinae* (61) is actually outnumbered by *C. borealis* (65) and is outnumbered 4:1 by *D. g. gallinulae* (see also Table 3). The absence of the starling from the May trappings slightly

exaggerates this effect since it removes one of the principal sources of *C. gallinae*. If the same calculation is made but excluding all residents and summer residents (the resident wheatears also excluded), and considering only passage migrants, the relatively low spring infestation rate for *C. gallinae* is still clearly demonstrated. In the spring *D. g. gallinulae* is four times as numerous as the other two species, both as regards infestations and numbers (see Table 1), and is the dominant flea. In the autumn (Fig. 1) the situation is reversed and *C. gallinae* becomes dominant.

Table 1*a*. *Fleas from spring and autumn migrants compared*

Three principal flea species	Fleas from spring-passage migrants	Fleas from autumn-passage migrants
D. g. gallinulae	151 inf., 267 spec., 39·8‰	5 inf., 6 spec., 0·8‰
C. gallinae	40 inf., 52 spec., 10·5‰	9 inf., 15 spec., 1·4‰
C. borealis	43 inf., 54 spec., 11·3‰	1 inf., 1 spec., 0·1‰

Table 2. *Total records of flea species by the month*

Species of flea	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Totals
D. g. gallinulae	1	143	42	169	53	16	5	1	430
C. gallinae	—	41	14	63	65	10	9	3	205
C. borealis	1	49	7	42	21	5	1	—	126
C. garei	—	3	—	—	1	—	—	—	4
C. vagabundus	—	—	—	—	1	—	—	—	1
C. fringillae	1	—	—	—	—	—	—	—	1

Table 3. *May trappings of the principal flea species*

	No. of specimens and infestations			
Species of flea	On all birds	On May trappings	On resident wheatears	On migrant wheatears
D. g. gallinulae	619 spec., 430 inf.	250 spec., 143 inf.	93 spec., 79 inf.	11 spec., 7 inf.
C. gallinae	329 spec., 205 inf.	61 spec., 41 inf.	56 spec., 47 inf.	4 spec., 3 inf.
C. borealis	154 spec., 126 inf.	65 spec., 49 inf.	75 spec., 57 inf.	12 spec., 9 inf.
Double and triple infestations	64	36	21	4
gallinulae/borealis	33	20	13	3
gallinulae/gallinae	21	11	3	1
gallinulae/garei	1	1	—	—
gallinae/borealis	4	1	3	—
Triple infestations	5	4	2	—

It would therefore appear that not only is the infestation rate for spring migrants enormously higher than for autumn migrants, but the relative number of the three principal species of flea is different at the different seasons. It seems probable that the main dispersal period of *D. g. gallinulae* and *C. borealis* is the spring and of *C. gallinae* the summer and autumn. Further evidence (discussed below, p. 393) suggesting a different main dispersal season for these fleas is obtained from studying the numbers of double and triple infestations with two or more species of fleas.

There may also be definite if slight host preferences, either by one or all three species of flea. Thus among passage migrants, wheatears and redstarts (see Table 4) are the favoured hosts of *C. borealis*. There is also some indication that the willow warbler and whitethroat are the preferred hosts (among the migrants) of *C. gallinae* in the spring. The most heavily infested group of spring migrant birds are the Turdidae (thrushes: 8 host species, 141 specimens) of which 61 % harboured fleas. The Sylviidae (warblers: 8 host species, 164 specimens) were also heavily infested with a 53 % spring rate. In both families *D. g. gallinulae* was the dominant flea, but in the case of the warblers this species was more numerous, not only with regard to infestations but specimens—practically two specimens per infestation (69:133). On the thrushes *C. borealis* greatly outnumbered *C. gallinae*, whereas among the warblers the reverse situation was found.

Table 4. *Host preference*

	D. g. gallinulae		*C. gallinae*		*C. borealis*	
	Inf.	Spec.	Inf.	Spec.	Inf.	Spec.
Willow warbler	16	29	**6**	10	2	3
Whitethroat	19	34	**8**	11	2	2
Sedge warbler	**15**	25	1	1	1	1
Passage migrant wheatears	7	11	3	4	**9**	12
Redstart	18	28	3	3	**9**	12
Robin	22	31	5	7	7	7

On the autumn migrants there are twelve infestations of Turdidae out of 322 examined and only two out of 155 Sylviidae—or 1 % compared with 3 % of the former.

We have remarked several times in the foregoing paragraphs that birds probably pick up fleas on their northward passage. But what proportion are collected after arrival on Fair Isle? Unfortunately the data available are so scanty that they do little more than suggest further possible lines of investigation.

In the collection of fleas from the bodies of British Birds made by Miss Clay and Colonel Meinertzhagen it was found that the ratio of *C. garei* to *C. gallinae* was 1:11 and to *D. g. gallinulae* 1:12 (Rothschild, 1952, p. 201). This collection mainly contained small Passerines of which twenty were of the same host species as the Fair Isle collection, not migrants but birds collected more or less at random at varying seasons throughout the years. It seems probable that this collection represents a fair sample of the three common bird fleas and the ratio in which they occur on Passerine birds on the mainland of Britain where all three parasites are breeding species. However, all the evidence at present available (Rothschild, 1955) suggests that *C. garei* is not a breeding species on Fair Isle and therefore the migrants found harbouring this species can be assumed to have brought them to the Island and not to have picked them up after arrival. From the spring passage migrants *C. garei* was recorded three times, or an 0·8 % infestation. Assuming that the Passerine migrants as a whole have a general infestation rate (with regard to the species ratio) comparable to that found on the Clay/Meinertzhagen collection (see above) one

would have expected eleven *gallinae* infestations for each *garei* or thirty-three in all. There were actually forty (excluding summer resident and resident hosts) suggesting that seven *gallinae* (16 %) were picked up on the Island after arrival. On this assumption we should expect about 36 *D. g. gallinulae* infestations on arriving migrants, but there were in fact no less than 144! (again excluding residents). The figure suggests that a huge proportion, about 75 %, are acquired after arrival. When we consider that the re-infestation rate of spring retraps with *D. g. gallinulae* is 55 %, this figure is well within the bounds of possibility.

Table 5. *Percentage infestation of migrants (selected from Table* 1)

Host	Percentage infested	
	Spring	Autumn
Common redstart	75·8	12·5
Sedge warbler	68·0	1 only examined
Blackbird	57·1	3·7
Willow warbler	53·8	1·4
Garden warbler	52·9	0
Robin	51·7	7·6
Whitethroat	42·1	0

Table 6. *Retraps of spring migrants*

	D. g. gallinulae	*C. borealis*	*C. gallinae*	Negatives
Willow warbler (*Phylloscopus t. trochilus*)	2 (4♀+1 lost)	—	—	4
Garden warbler (*Sylvia borin*)	1 (1♂, 1♀)	—	—	—
Whitethroat (*S. communis*)	2 (1♂, 4♀)	—	—	2
Redstart (*Phoenicurus phoenicurus*)	2 (2♀)	—	—	1
Robin (*Erithacus rubecula*)	3 (1♂, 2♀)	—	—	Nil
Blackbird (*Turdus merula*)	—	—	—	1
Total	10 positive (17 specimens)	Nil	Nil	8

Stansfield (1957) made the fascinating discovery that on Skokholm *C. gallinae* was absent from the nests of the wheatear (7), rock pipit (3), carrion crow (3), raven (1), skylark (1), and manx shearwater (22). Furthermore, it was not apparently present on the spring migrants. The exact number of the birds examined is not stated, but 11 were positive for *D. g. gallinulae* on spring passage. The infestation rate of *C. gallinae* on Fair Isle was 10 % of all spring migrants and these figures of Stansfield, although too small to be conclusive, are quite suggestive. In spring 1955 he examined 11 whitethroats—all negative for *C. gallinae*, whereas out of 33 examined on Fair Isle 8 (or 1 in 4) were positive for this species. Stansfield also failed to find *D. g. gallinulae* in the nests of the birds he examined on Skokholm. Since this species is usually found only in relatively small numbers in nests it is more easily overlooked. However, the combined rate of infestation with *D. g. gallinulae* of the spring migrant sedge warbler and whitethroats on Skokholm was 28 % (8 positive out of 28 examined), but on Fair Isle 41 % (34 positive out of 82

examined). This suggests that the additional 13 % infestation rate of this species on Fair Isle could represent the fleas picked up by them after arrival.

One can invisage the migrants gradually becoming more and more heavily infested as they move northwards, bad weather or any other factor which lengthens their sojourn on an Island such as Ushant or Fair Isle increasing the infestation rate particularly with *D. g. gallinulae*.

Table 7. *Infestations of Turdidae and Sylviidae compared*

	D. g. gallinulae		*C. gallinae*		*C. borealis*	
	Inf.	Spec.	Inf.	Spec.	Inf.	Spec.
Turdidae (thrushes)						
8 species, 141 birds, 85 positive, 61 % spring infestation rate (spring)	61	91	12	15	32	39
8 species, 322 birds, 12 positive, 3·5 % autumn infestation rate (autumn)	3	3	7	11	1	1
Sylviidae (warblers)						
8 species, 164 birds, 84 positive, 53 % spring infestation rate (spring)	69	133	20	28	5	6
13 species, 155 birds, 2 positive, 1·2 % autumn infestation rate (autumn)	2	2	—	—	—	—

Since *C. borealis* is a species confined to outlying western islands and a few moorland districts on the mainland we should be able to hazard a better guess with regard to the number of birds picking up this species after arrival. But unfortunately we do not know the route followed by the migrants; if, for example, they pass through the Island of Ushant (Rothschild, 1948) on their passage northwards or feed along some moorland coastal strip, they might acquire *C. borealis* before reaching Fair Isle. In September only 1·9 % of the resident wheatears were found to harbour *C. borealis* on their bodies, whereas in May their infestation rate was 22·8 % (see Table 10, less 1953 records and Fig. 5). It would come as no surprise to me if subsequent knowledge concerning the hosts migration route indicated that this additional 21–22 % were all acquired on Fair Isle. It seems very likely that the warblers acquire this species either on Fair Isle itself or on some other bottle-neck on their northward passage. These hosts are not favoured by *C. borealis* which has not been found breeding in their nests.

DOUBLE AND TRIPLE INFESTATIONS

As we have already pointed out, apart from those birds which harboured one or more specimens of a flea, a number of resident and migrant birds were infested with two or more species. These double infestations (see Tables 3 and 8) present certain curious features which cannot be satisfactorily explained.

The number of fleas per bird (see Tables 14 and 15) is not distributed purely by hazard. If hosts and fleas were uniformly scattered over the island one would

expect to find one flea per infested bird. As we have seen, this is not the case. The mean per infested spring migrant is 1·9. As far as fledglings are concerned such a distribution is expected since an infested nest can harbour many hundreds of fleas and an uninfested nest is devoid of any. However, the spring migrants arrive before the nests of the year are built. These facts suggest that the fleas which are picked up on the island (or elsewhere) away from any nest are also somewhat concentrated in their distribution in space, so that a bird passing within reach of one flea is liable to pick up another.

Table 8. *Distribution of double infestations by the month*

	May	June	July	Aug.	Sept.	Oct.	Nov.	Totals
D. g. gallinulae + *C. borealis*	20	2	7	4	—	—	—	33
D. g. gallinulae + *C. gallinae*	11	4	5	—	—	—	1	21
D. g. gallinulae + *C. garei*	1	—	—	—	—	—	—	1
C. borealis + *C. gallinae*	1	—	1	1	1	—	—	4
D. g. gallinulae + *C. borealis* + *C. gallinae*	4	1	—	—	—	—	—	5
Totals	37	7	13	5	1	—	1	64

The double infestations not only emphasize this aspect of the phenomena but suggest that one combination of species, namely *D. g. gallinulae* with *C. borealis*, occurs more frequently than is to be expected if these were purely chance associations. When the number of separate *C. gallinae* and *C. borealis* infestations is considered it will be appreciated that only four double infestations with these two species is a comparatively very small number. Furthermore, three of these infestations occurred on the resident wheatears. It will be seen (Table 11) that out of fourteen nests of the wheatear examined, all harboured *C. borealis*, which was the dominant species, thirteen harboured *D. g. gallinulae*, and seven *C. gallinae*. One would expect that most double infestations would involve *C. borealis* with *D. g. gallinulae*, followed by *C. borealis* with *C. gallinae*—there being a huge excess of *C. borealis* specimens over *D. g. gallinulae* in each nest. However, the double infestations of *C. borealis* with *C. gallinae* and *C. borealis* with *D. g. gallinulae* are both three in number.

When we come to consider the fleas on the bodies of the hosts in the whole collection this effect is even more marked (Table 3). Assuming that the fleas which the birds have acquired in the open have migrated or spread from some vacated nest (see Waterston, 1910; Ioff, 1941; Rothschild & Clay, 1952), we can partly account for this strange distribution by three assumptions:

(*a*) That fleas are liable, even when they have left the nest, to be concentrated and therefore one bird may often pick up more than one flea from one such concentration.

(*b*) That the period of most intense and active dissemination and closest association with the body of the host occurs chiefly at definite (but different) times of the year for *C. gallinae* and *C. borealis*, the latter occurring in the spring (probably overwintering in the cocoon) and the former in the late summer and autumn (with a proportion probably hibernating in the imaginal stage).

Table 9. *Infestation of principal resident birds*

Species of birds	Wheatear	Starling	Twite	Rock pipit	Meadow pipit	Crossbill	Totals
Total examined	1417	458	241	1076	532	120	3844
Number positive and % infested	162* (9·7 %)	82* (17·1 %)	11* (3·8 %)	161* (13·5 %)	56* (9·7 %)	5* (4·0 %)	477
No. positive for *D. g. gallinulae* and no. of specimens and sex	79:93 — 39 ♂, 54 ♀	8:8 — 3 ♂, 5 ♀	9:11 — 6 ♂, 5 ♀	124:166 — 78 ♂, 88 ♀	46:60 — 28 ♂, 32 ♀	4:4 — 1 ♂, 3 ♀	270:342 — 155 ♂, 187 ♀
No. positive for *C. gallinae* and no. of specimens and sex	47:56 — 15 ♂, 41 ♀	74:159 — 64 ♂, 95 ♀	2:2 — 1 ♂, 1 ♀	19:21 — 8 ♂, 13 ♀	5:5 — 1 ♂, 4 ♀	1:2 — 1 ♂, 1 ♀	148:245 — 90 ♂, 155 ♀
No. positive for *C. borealis* and no. of specimens and sex	58:75 — 42 ♂, 33 ♀	1:1 — 1 ♂	Nil	19:19 — 8 ♂, 11 ♀	3:3 — 3 ♀	Nil	81:98 — 51 ♂, 47 ♀
No. of negative and positive retraps	39 negative, 7 positive	11 negative, 11 positive	18 negative, 2 positive	222 negative, 37 positive	49 negative, 7 positive	?	339 negative, 64 positive (15·8)
Sex of infested bird (when known)	17 ♂, 10 ♀	10 ♂, 7 ♀	2 ♂, 2 ♀	3 ♂, 2 ♀	1 ♀	4 ♂, 1 ♀	36 ♂, 23 ♀
Infestations with two or more species	21	2	Nil	2	Nil	Nil	25

* 1953 specimens included but adjustment made in the percentages; one sparrow omitted from retraps.

(*c*) That the period of dissemination for *D. g. gallinulae* is also mainly in the spring and early summer (dropping off sharply in August), but over a more extended period than is the case with *C. borealis* and that its association with the body of the host is altogether more prolonged and closer than with the other species involved.

It must be emphasized, however, that all five triple infestations occurred in the spring (Table 8) and some dissemination of *C. gallinae* must occur at this period too, possibly by fleas which have over-wintered in the imaginal stage.

THE SEX OF INFESTED BIRDS

The sex was recorded of 103 of the migrants positive for fleas and much to my surprise it was found that nine more male than female birds were infested. A slightly greater excess of infested males was found in the resident birds (37 infested males to 23 infested females) or a total of 90 infested males (57 %) to 70 infested females (43 %). Unfortunately, the sex of relatively few negative birds is recorded on my slips, the majority bearing the note 'migrant adult'. In 1952 and 1954 the sex of 125 males and 107 females was recorded in all, of which 58 (46 %) males and 41 (38 %) females were infested. There are unfortunately no records of the sex of the negative birds in either 1953 or 1955. In view of the greater share the female so often takes in sitting on the eggs or brooding the young (in nests which are frequently pullulating with fleas) I fully expected to find the female with a higher infestation rate than the male. Certainly this is not the case with the Fair Isle migrants. Among the double and triple infestations in which the sex of the host was known, 20 were on male birds and only 14 on females, and, furthermore, the mean number of fleas per infested male bird is 2·5 and that of the female only 1·8. This applied to residents as well as migrants. With this in mind Williamson's observation (personal communication) that many first-year male wheatears explore a number of old nesting holes before settling down, is of interest. Migrant wheatears also examine holes and burrows on their way through Fair Isle.

SEX RATIO OF THE FLEAS

The sex ratio (see Fig. 2) of *D. g. gallinulae* for the 430 records was 294 males to 325 females or an excess of 31 females. It was pointed out previously (Rothschild, 1952) that the proportions of the sexes of *D. g. gallinulae* recorded from the bodies of birds were about equal, but that there was a very big preponderance of females recorded from nests. A curious feature of the sex ratio of this flea is that during May and June there is an excess of males on the host (see Fig. 2A). Thus in May there were 131 males to 119 females, and in June 39 males to 33 females, and on spring-passage migrants only (Table 1), 139 males to 128 females. By July on the resident birds the situation is reversed and there is an excess of 34 females (124) over the males (90), and in September (Table 1) on the autumn migrants the ratio is 0 male to 6 females. In the nest of the wheatear (Tables 11 and 11*a*) we found the usual excess of females which were approximately double the number of males. However, on the body of this host in May and June there was a slight excess of

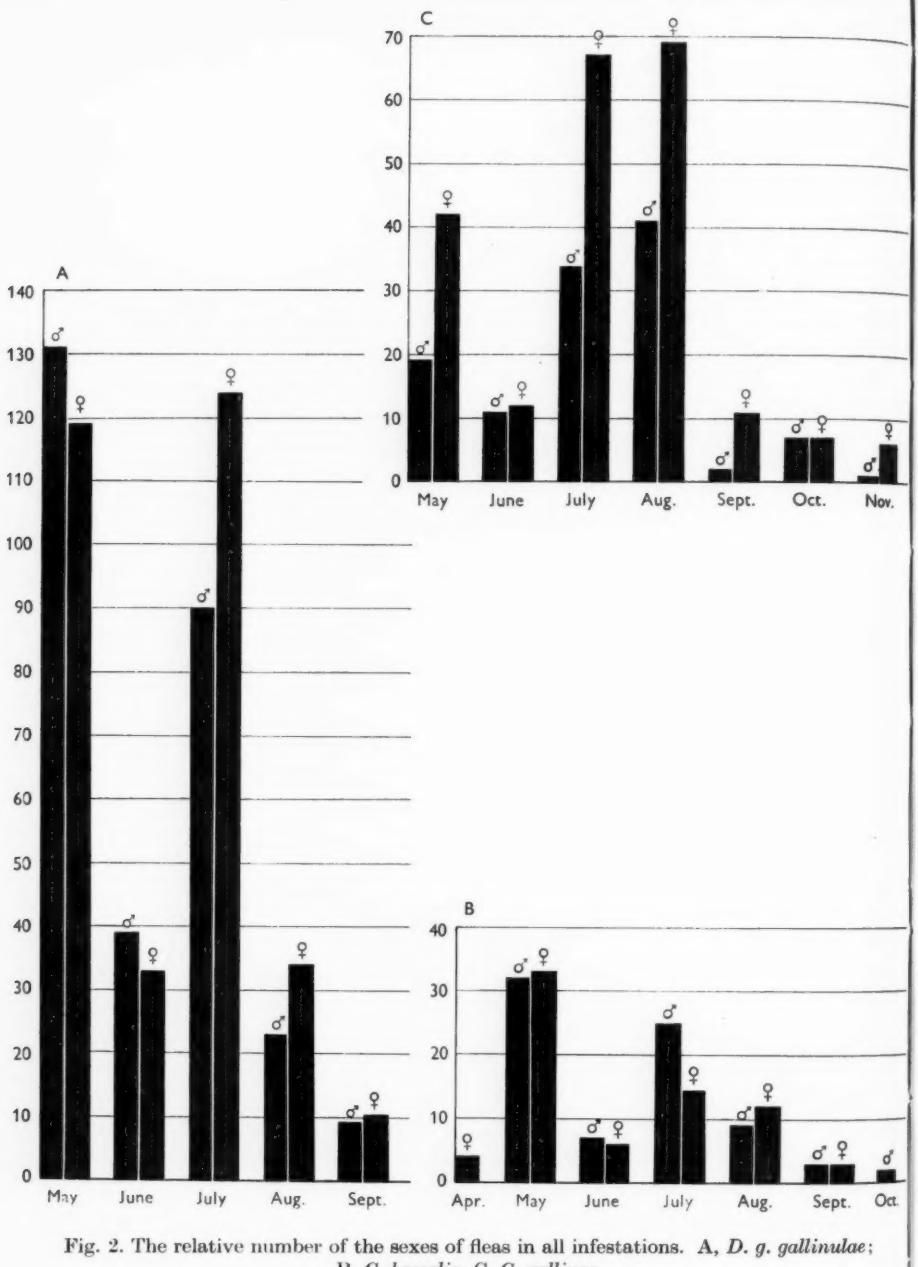


Fig. 2. The relative number of the sexes of fleas in all infestations. A, *D. g. gallinulae*; B, *C. borealis*; C, *C. gallinae*.

males (14 males, 12 females) and it was not until July that females began to exceed the males.

The proportion of male to female *C. borealis* (see Fig. 2 B) was exactly in the ratio 1:1 (77 males, 77 females) and was level throughout the 6 months on residents and migrants alike. In the nest of the wheatear (Table 11), however, there was a big excess of females (551 males to 786 females).

It was also noted previously (Rothschild, 1952) that both in the nest and on the bodies of the host female *C. gallinae* outnumbered the males 3:2. In the case of the Fair Isle birds, including the wheatear, the ratio was found to agree with this figure (115 males to 214 females), but in the nest of the wheatear the proportion of the sexes of *C. gallinae* (48 males to 50 females) was most unusual. On the birds in May (Fig. 2C) the females definitely outnumber the males 2:1 (19 males to 42 females and on the spring migrants 14 males to 38 females), and on autumn migrants there are 4 males to 11 females recorded. Therefore, in the case of both *C. gallinae* and *D. g. gallinulae* the autumn migrants carry a higher proportion of female to male fleas. The high rate of male *D. g. gallinulae* in the spring (see Fig. 2 A) is a very curious feature of the migrant infestations.

THE RESIDENT BIRDS

The five or six species of resident birds with which we are chiefly concerned (Tables 9, 12 and 13) present several interesting problems relating to the distribution of the three commonest species of Fair Isle bird fleas.

Unfortunately, despite the fact that over 3000 of these resident birds (chiefly pertaining to 4 species) were 'de-fleaed', there are some peculiarly exasperating gaps in the collecting. Even by pooling all the years for which negative records (Table 10) were available for comparison we cannot get a really satisfactory picture of the breeding cycle of these common bird fleas. There is, for example, a great dearth of records in May and June from resident birds. At this moment the rush of migrants is over, the easily trapped young are still in the nest, while the adult resident birds do not enter the traps at this season.

If we consider the infestation rate on the bodies of the principal resident birds, we find a more varied picture than we obtained from a comparison of spring and autumn migrants. The case of *C. borealis*, however, is fairly clear-cut. This species both on the rock pipit (but note the paucity of records) and the wheatear (Fig. 7) has a peak in May and a rapid fall thereafter. The rock pipit is a permanent resident, double-brooded and commences nesting 3 weeks before the wheatear which is single-brooded, and a summer resident only. The peak period for association with the host is not therefore closely linked with the breeding cycle of that year. If all the 80 *C. borealis* records from residents are pooled, i.e. the odd records for the starling and meadow pipit also included, approximately the same curve is obtained (see Fig. 8).

In the case of *D. g. gallinulae* there is a high May rate but a sharp rise in June for the three host species for which figures are available (see Fig. 3), followed by an even steeper fall in July and August. We must stress once again that the numbers

of examinations in June are inadequate, but nevertheless the figures for the pipits* (12 positive out of 14 examined) suggest a very high rate in that month. These infestations were obtained chiefly from juvenile birds which by the following month have a greatly reduced infestation rate.

Table 10. *Total infestations for* 1951, 1952, 1954 *and* 1955 *May to November of principal resident birds*

Hosts	May	June	July	Aug.	Sept.	Oct.	Nov.	Totals
Wheatear								
D. g. gallinulae	5	3	50	10	—	—	—	68
C. gallinae	3	1	19	14	3	—	—	40
C. borealis	8	3	27	7	3	—	—	48
Negative records	24	14	329	681	203	4	—	1255
Starling								
D. g. gallinulae	—	2	4	1	—	—	1	8
C. gallinae	1	9	25	28	3	2	2	70
C. borealis	—	—	—	1	—	—	—	1
Negative records	2	51	156	84	29	54	—	376
Rock pipit								
D. g. gallinulae	5	5	71	28	8	1	—	118
C. gallinae	—	—	6	5	0	1	—	12
C. borealis	1	—	9	4	1	—	—	15
Negative records	5	1	292	470	130	17	—	915
Meadow pipit								
D. g. gallinulae	3	7	24	4	4	—	—	42
C. gallinae	—	—	2	3	—	—	—	5
C. borealis	—	—	2	1	—	—	—	3
Negative records	5	6	134	219	82	30	—	476
All hosts (including 1953 records)								
D. g. gallinulae	143	42	169	53	16	5	1	429
C. gallinae	41	14	63	65	10	9	3	205
C. borealis	49	7	42	21	5	1	—	125
Negative records of bird species from which fleas have been recorded at least once	160	92	972	1643	491	138	4	4078

In the case of the *C. gallinae* the picture is more varied. The peak infestation on the wheatear is also in May (Fig. 5) with a fairly steady fall onwards, but so much less rapid than the fall in *D. g. gallinulae* that *C. gallinae* becomes the dominant flea on this bird in August (see Figs. 4 and 6), as well as among the residents as a whole (see Fig. 1). On the starling (Fig. 9), however, which is the principal host of *C. gallinae*, there is a very sharp rise in August when the second-brood young are coming into the trap. This is a different pattern from that found for either *D. g. gallinulae* or *C. borealis*, and suggests that for the starling at any rate the main dispersal period is in the summer, closely linked with the breeding cycle of that year. It is very unfortunate that we only trapped 3 starlings in May as this leaves us in doubt as to whether or not there is an early spring peak for this species.

* I examined five nests of the meadow pipit collected in June after the young had flown but all were negative for fleas.

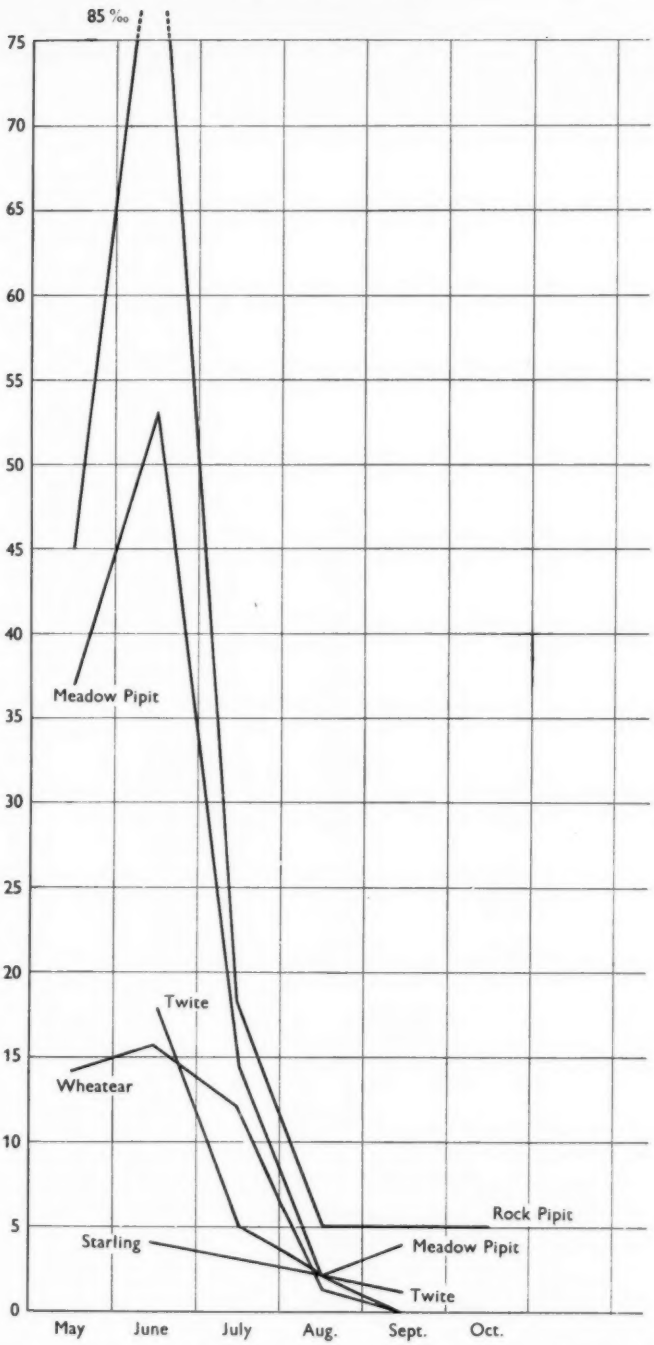


Fig. 3. ***D. g. gallinulae.*** **The percentage infestation of resident birds.**

In 1955 Williamson calculated the number of fleas per 100 starlings examined at weekly periods from 23 June until 1 September (see Fig. 10). The two peaks coincided with the peaks of the first- and second-brood young coming into the traps. Unfortunately, the data available does not permit me to make a similar calculation for other years and whether or not there was an earlier but smaller peak in 1952–54 which is correlated with the first-brood young I cannot say. There is, however, considerable variation from year to year in the degree of infestation of the second-

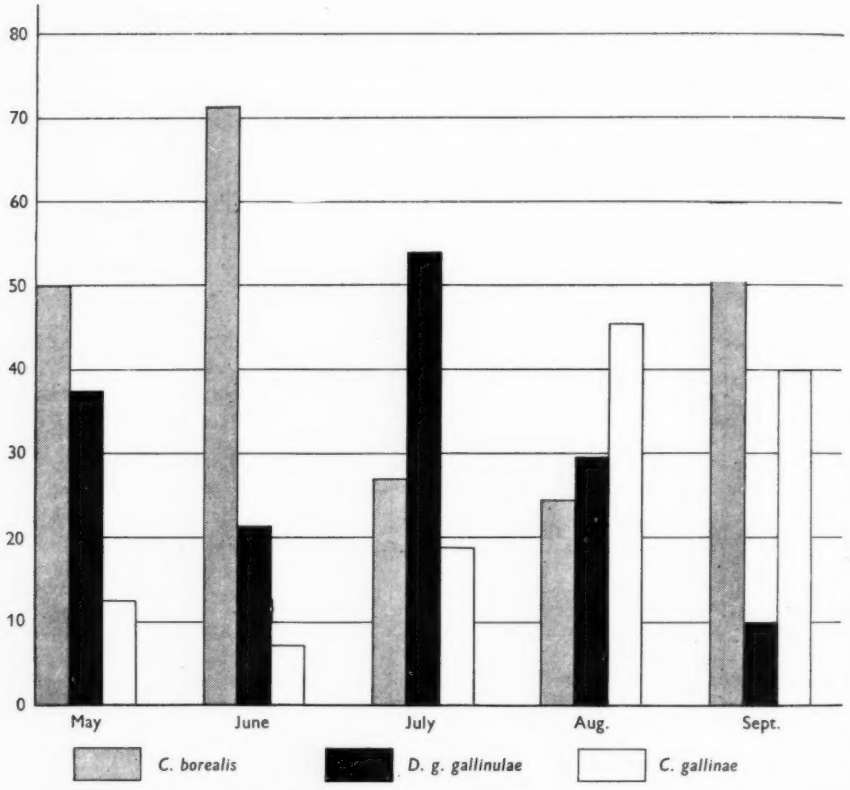


Fig. 4. The dominant species of flea among infestations of the resident wheatears. Percentage infestation per month (number of specimens not infestations, including those for 1953).

brood young. Thus in 1954 infested starlings taken in the traps between 24 June and 20 August had a mean of 1·1 flea per bird, whereas in 1952 the mean was far higher, 4·7 per infested bird (see Table 15). The percentage infestation in the latter year was also twice as high as in the former.

We have said already (p. 390) that there are indications that fleas show some host preference among the migrant birds, but this becomes considerably clearer in the case of the breeding resident Passerines. We have also mentioned how spring migrants 'de-fleaed', released and subsequently retrapped and re-examined are

found to have acquired numerous specimens of *D. g. gallinulae* while on the Island. The same phenomena can be observed with juvenile resident rock pipits and wheatears during July and August. Retraps of the former species have acquired 26 re-infestations (34 specimens) of *D. g. gallinulae* and the latter 5 re-infestations (6 specimens). The starling, however, acquired only 1 specimen of *D. g. gallinulae*, but 10 infestations (22 specimens) of *C. gallinae* (see Tables 11 and 12). As we have said (p. 385), these young birds—wheatears, starlings and pipits—all feed over the same ground and in order to get into the traps must to some extent associate. It

Table 11. *Flea fauna of wheatear's nests*

Nest, date of collection ...	21. vi. 52		21. vi. 52		22. vi. 52		26. vi. 52		30. vi. 52		30. vi. 52		3. vii. 51		3. vii. 51	
Location Fair Isle (all with young fledged) ...	Cavity under a boulder		Burrow in peat bank (Wirrie)		Homisdale		Sandy burrow (North Haven)		Burrow in air strip		Burrow in cliff edge		No data		In a wall	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
C. borealis	3	5	19	10	2	3	7	3	3	6	—	1	2	—	58	71
C. gallinae	—	—	12	17	1	—	1	1	9	3	1	—	—	—	—	—
D. g. gallinulae	—	1	1	2	1	1	7	8	—	—	—	1	—	1	1	—

Nest, date of collection ...	3. vii. 51		3. vii. 51		3. vii. 51		3. vii. 51		Sept. 1954		Sept. 1954		Totals	
Location Fair Isle (all with young fledged) ...	Under stones		In burrow		Nest in lighthouse road		In gully behind trap		No data		No data		14	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
C. borealis	153	167	27	35	21	21	25	35	150	275	81	154	551+786 = 1337	
C. gallinae	—	—	—	—	1	2	—	—	—	—	23	27	48+50 = 98	
D. g. gallinulae	3	10	9	12	2	3	—	1	1	1	1	—	26+41 = 67	

Table 11*a*. *Fleas in nest and on body of wheatear compared*

Total no. wheatears examined: 1417 (collected April–October)

	Inf.	Spec.	Males	Females
Positive for fleas: 162 (9·7 %)*				
D. g. gallinulae	79	93	39	54
C. gallinae	47	56	15	41
C. borealis	58	75	42	33

Total no. nests examined: 14 (collected June–September)

	Inf.	Spec.	Males	Females
Positive for fleas: 14 (100 %)				
D. g. gallinulae	13	67	26	41
C. gallinae	7	98	48	50
C. borealis	14	1337	551	786

appears that in a case like this the imago of *D. g. gallinulae* does not find the starling attractive and therefore does not jump on to a bird of this species moving in its vicinity, or, more probably, once having reached it soon leaves it again. It seems hardly possible that the habits of young starlings are so different from the other young birds on Fair Isle that after leaving the nest, and despite feeding together, they are kept from any contact with this species, while young wheatears and rock

* 1953 specimens included but adjustment made in the percentages.

pipits are constantly brought within range. Nevertheless, the birds' specialized behaviour, i.e. associating in close flocks and roosting communally on cliffs or in caves, brings them into contact with dispersing *C. gallinae* far more frequently than any other bird on the Island.

In a previous paper (Rothschild, 1952) some factors possibly affecting the distribution of the common bird fleas were discussed. It was suggested that the humidity requirements of the larval fleas affected the survival of a given species within certain nests; for instance, conditions in a starling's nest were relatively dry and

Table 12. *Retraps of breeding species by the month for* 1952, 1953, 1954 *and* 1955

Host	May	June	July	Aug.	Sept.	Oct.	Totals Pos.	Totals Neg.
Wheatear (*Oenanthe oenanthe*)								
D. g. gallinulae	—	—	3♂, 2♀	1♂	—	—	5 } 7	—
C. gallinae	—	—	1♀	2♀	—	—	2 }	—
Negative retraps	3	1	10	24	1	—	—	39
Rock pipit (*Anthus spinoletta*)								
D. g. gallinulae	1♀	—	5♂, 7♀	10♂, 8♀	1♂, 2♀	—	26 } 38	—
C. gallinae	—	—	2♂, 1♀	1♂, 3♀	—	1♂	6 }	—
C. borealis	1♂	—	1♂, 1♀	2♂, 1♀	1♀	—	6 }	—
Negative retraps	—	—	28	104	54	36	—	222
Meadow pipit (*Anthus pratensis*)								
D. g. gallinulae	—	3♂	2♂, 4♀	—	—	—	5 }	—
C. gallinae	—	—	1♀	—	—	—	1 } 7	—
C. borealis	—	—	1♀	—	—	—	1 }	—
Negative retraps	—	—	9	35	5	—	—	49
Twite (*Carduelis flavirostris*)								
D. g. gallinulae	—	—	1♂, 3♀	—	—	—	2	—
Negative retraps	—	1	5	7	—	5	—	18
Starling (*Sturnus vulgaris*)								
D. g. gallinulae	—	—	1♂	—	—	—	1 } 11	—
C. gallinae	—	—	1♀	11♂, 10♀	—	—	10 }	—
Negative retraps	—	1	1	8	1	—	—	11
Wren (*Troglodytes troglodytes*)								
D. g. gallinulae	—	—	—	3♀	—	—	1	—
Negative retraps	—	—	—	—	—	—	—	—
Totals: Positive	2	1	27	31	4	1 }	66	339
Negative	3	3	53	178	61	41 }		

therefore favoured *C. gallinae* in competition with *D. g. gallinulae*. Bates (1956) put more importance on competition between fleas within the nest, showing that both these species can survive in wet or dry nests but that larvae of *D. g. gallinulae* are greatly reduced in numbers or entirely eliminated from both types of nest when larvae of *C. gallinae* are present in the nest. Probably both Bates and myself underestimated the selective role (direct or indirect) played by the adult flea in the case of the starling at least, and probably in other species such as the swallow. Before competition for food between larvae can begin, or dry or wet nesting conditions favour one at the expense of the other, the species has got to reach the nest

in question! In the case of the starling* it seems relatively uncommon that any species of flea except *C. gallinae* does in fact reach the nest, since (see above) these birds have few infestations of *D. g. gallinulae* or *C. borealis* on their bodies despite the fact that on Fair Isle they must be constantly exposed to infestations of both species. Among the resident birds the starling is the most heavily infested species from the point of view of numbers of specimens per bird (Table 15). A larger proportion of birds had more than one flea—the mean was around 2·4 fleas per infested bird—compared with 1·2 for the pipits and 1·3 for the resident wheatears.

The twite (Table 9) is an outstanding bird among the breeding Passerines since it is the least parasitized of all species taken in the traps with an over-all rate of only

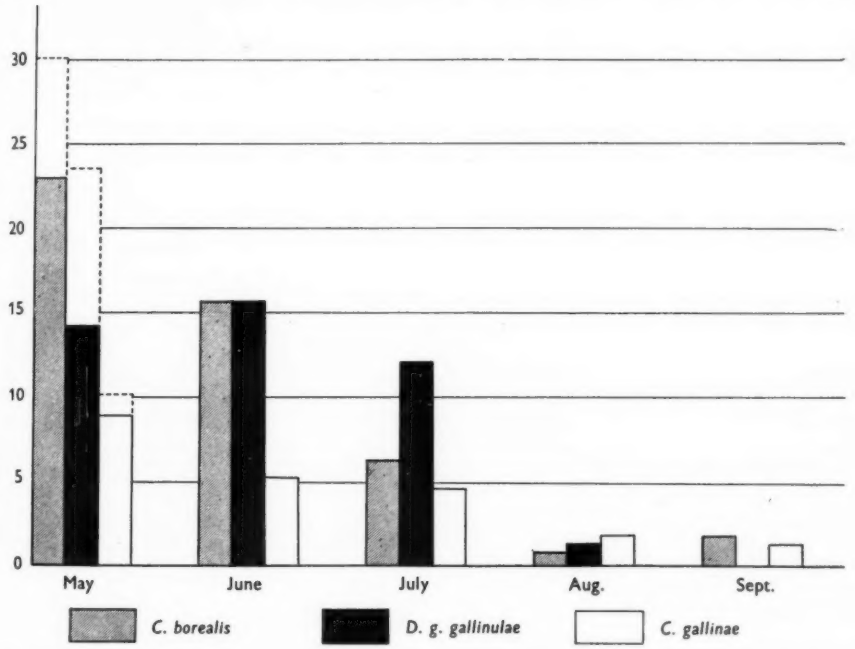


Fig. 5. Resident wheatear: percentage infestation. (The dotted lines indicate the percentage if the spring passage migrant wheatears are included; 1953 records excluded.)

3·9 %. The twite apparently does not (Corbet, 1956) return to old nesting sites, and furthermore builds very open rather flimsy nests, but since it feeds over the same ground as the other resident species and associates in flocks in the autumn, a somewhat similar infestation rate could be expected. The simplest explanation is that this bird is not attractive to fleas, and, consequently, rarely acquires these insects or retains them for only short periods.

The difference between the flea fauna of the rock pipit and meadow pipit can be accounted for partly by the difference in their nesting habits (see p. 384). Both birds (Tables 9 and 10) are infested with the three common Fair Isle species, but

* I found 2 *D. g. gallinulae* in a starling's nest collected on Fair Isle in 1954.

the meadow pipit is primarily a '*gallinulae*' host and only under 1 % harbour *gallinae* or *borealis*. The paucity of records of *C. gallinae* compared with those from the rock pipit suggests some definite host selection on the part of the adult flea. The rock pipit, which returns to old nesting sites, is more heavily infested with an overall rate of 13·5 % as against 9·7 % for the meadow pipit.

Three interesting facts about the fleas of the resident wheatear have been noted. First, the proportion of *D. g. gallinulae*, *C. gallinae* and *C. borealis* on (*a*) the bodies of these birds, and (*b*) in their nests, is strikingly different. Secondly, the wheatear (Table 12) seems to re-acquire *D. g. gallinulae* (5 positive retraps) in the open more readily than *C. borealis* since 46 retraps, 3 of which were examined in May and June (the peak period for *C. borealis* on Fair Isle), were all negative for this species. Thirdly, wheatears (Table 3) showed a high proportion of double infestations with two species—*D. g. gallinulae* and *C. borealis*.

On the body of the host *D. g. gallinulae* outnumbers *C. borealis* in about the ratio 7:5, but in the nest the situation is completely reversed and *D. g. gallinulae* is outnumbered 19:1. Twelve of these nests were kept for only a few weeks after collecting, but from the two examined over a period of 6 months 449 *C. borealis* hatched out and the *borealis*:*gallinulae* ratio was 32:1. These facts confirm the suggestion (Rothschild, 1952) that *D. g. gallinulae* is more closely connected with the body of the host and less of a 'nest' species than any of the other common bird fleas. It may also rely more on wide dissemination and finding a host in the open (i.e. away from the nest). This is suggested by its dominance on retraps as well as the dominance in the collection as a whole. Another point worth noting in connexion with the ratio of fleas in the nest and on the body of the host is that in August *C. gallinae* becomes the dominant flea (see Fig. 4) on the body of the wheatear at a period when it is greatly outnumbered by *C. borealis* in the nests we examined.

To sum up, one can say that the study of fleas found on the resident birds tends to support to some extent the suggestion that the three species concerned have different periods in which they become more closely associated with the body of the host. This applies particularly to *C. borealis* with a May peak and *C. gallinae* (on the starling) with an August peak of activity.

Unfortunately, very little work has been done on the cycle of fleas in the nest, although the literature abounds with casual observations. Thompson (1954) kept a number of wheatear nests in a cool room during the autumn and winter and claims that almost the total emergence of *C. borealis* occurred in the spring. Jordan & Rothschild (1910) found *D. g. gallinulae* emerged from a dipper's nest in small numbers throughout the year. I have observed hundreds of *C. vagabunda alpestris* hatch from cocoons in chough's nests immediately after the young had flown. Nordberg (1936) in Finland found *C. gallinae* most abundant (in the nest) about the time the fledglings left and that the fleas migrated from the nest when the temperature fell. Williamson examined a few starlings' nests for me on Fair Isle and was surprised to find a great dearth of fleas. He suggested they must have all been carried away on the second brood of fledglings. A general dispersal from the nests seems more probable when the re-infestation rate is considered. The conditions, however, in a nest which is first disturbed when collected and then kept in a box or

bag indoors are so different from the natural conditions that no great importance can be placed on such observations. Hatching in these circumstances may bear little, if any, relation to the cycle in nature. Prolonged and carefully controlled experiments are required.

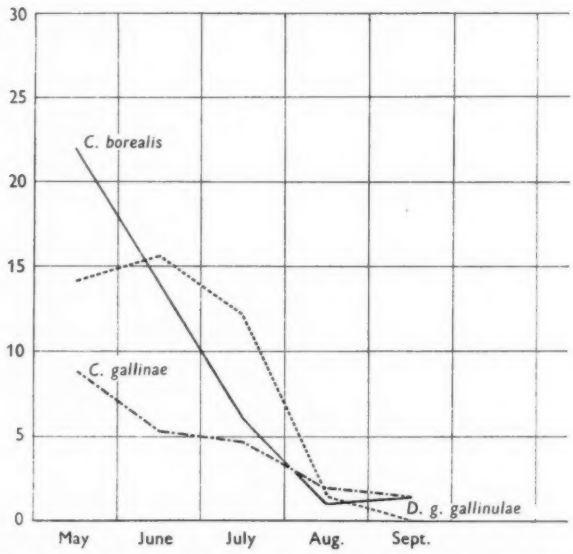


Fig. 6. Resident wheatear: percentage infestation by the month with the three principal species of flea.

THE RETRAPS

Undoubtedly the most interesting part of Williamson's collecting concerns the retraps. As far as I know this is the first time that a considerable number of birds have been trapped, 'de-fleaed', ringed, released, retrapped and again 'de-fleaed'. It is also the first time we have positive evidence that fleas are picked up by birds before the breeding season commences, away from the nest, and during migration. The number of bird fleas which have been found 'free' and in situations far from any nests (Rothschild, 1952) and also on mammalian hosts suggested that this probably occurred.

The outstanding feature of the retraps (see Tables 6 and 12) is the high proportion of spring migrants (55%: 10 out of 18) which acquired fleas after arrival. Eleven out of the 12 May/June infestations were with *D. g. gallinulae* (19 specimens). The single May *C. borealis* was found on a resident rock pipit. Since 55 *C. gallinae* infestations were recorded among our original May and June trappings (see Table 2) we expected 2–3 infestations among the retraps during these months, but no birds retrapped before July were found to have acquired *C. gallinae*. Another curious feature is the apparent scarcity of *C. borealis* on the retraps, particularly when one considers that infested nests of the wheatear were situated a few yards from the

Table 13. *Infestation rate: comparison of resident birds with retraps of resident birds*

(Excluding one 1953 record.)

Species of bird	Wheatear		Rock pipit		Meadow pipit		Starling		Twite	
Percentage infested all birds examined	9·7		13·5		9·7		17·1		3·9	
Total retraps examined	46		259		56		22		20	
Positive retraps and percentage infested	7 15·2%		37 14·3%		7 12·5%		11 50%		2 10%	
	All birds	Retraps	All birds	Retraps	All birds	Retraps	All birds	Retraps	All birds	Retraps
Percentage *D. g. gallinulae* infestation all birds and retraps compared	4·8	10·9	11·4	10·0	8·0	9·0	1·7	4·5 (1 retrap)	2·9	10
Percentage *C. borealis* infestation all birds and retraps compared	3·3	Nil	1·4	2·7	0·6	1·8 (1 only)	0·2	0	Nil	Nil
Percentage *C. gallinae* infestation all birds and retraps compared	2·8	4·3	1·1	2·3	0·9	1·8	15·4	45·4	0·8	Nil

Heligoland traps. The figures are, however, very small in the spring (when there is apparently a peak period for *C. borealis* on the host) and insufficient for our purpose.

The sharp drop in August of *D. g. gallinulae* on the body of the host is shown exceptionally well on the retraps. In July 26 % of all retraps acquired this species, but in August the infestation rate dropped to 7 %. This was also apparent when a 'good' *gallinulae* host such as the rock pipit is considered, separately as well as in

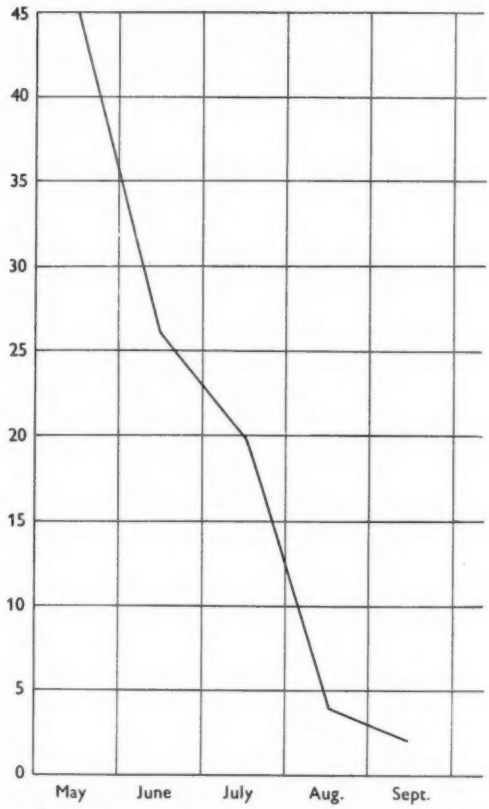


Fig. 7. Resident wheatear: infestation with all fleas per month. *D. g. gallinulae*, 43 %; *C. borealis*, 31 %; *C. gallinae*, 26 %.

over-all figures. Thus in July, 9 out of 40 retraps of this species acquired *D. g. gallinulae*, but in August, only 13 out of 123.

All the residents show a higher re-infestation rate than the original trap rate (see Table 13), one of the curious aspects being that in the case of the wheatear the re-infestation rate with *D. g. gallinulae* is 2 % higher than in the original infestations—a larger increase than for either of the pipits which are more definite *gallinulae* hosts.

Some of the migrants acquired their fleas fairly rapidly—for instance the whitethroat first examined negatively for fleas at 7·45 a.m. on 10 May had acquired 3 specimens of *D. g. gallinulae* exactly 23 hr. later when it re-entered the trap. Quite a few of the migrants lingered on the Island several days. Unfortunately a willow warbler caught on 6 May was not 'de-fleaed', but when retrapped on 10 May it was infested with 1♀ *D. g. gallinulae* and 2♀ *C. borealis*, which it had had ample time to acquire; it was noted that numbers of willow warblers spent the day of 6 May feeding pipit-like on the close-cropped grass in a warm drizzle. At the other

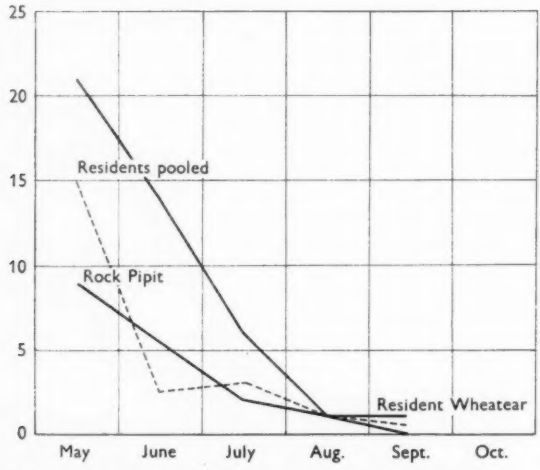


Fig. 8. *C. borealis*; percentage infestation of resident wheatear, rock pipit and all residents pooled.

Table 14. *Number of fleas per bird*

(1952, 1954, 1955 records.)

	All birds	Spring migrants	Retraps
Number of birds with 0 fleas	3882	291	352
Number of birds with 1 flea	404	82	50
Number of birds with 2 fleas	101	49	12
Number of birds with 3 fleas	48	20	10
Number of birds with 4 fleas	16	6	3
Number of birds with 5 fleas	4	4	—
Number of birds with 6 fleas	5	3	—
Number of birds with 7 fleas	3	—	—
Number of birds with 8 fleas	1	—	—
Number of birds with 9 fleas	1	1	—
Number of birds with 10 fleas	1	—	—
Number of birds with 11 fleas	1	—	—
Number of birds with 20 fleas	1	—	—
Number of birds with 22 fleas	1	—	—
Number of fleas per bird:	0·2	0·7	0·3
Number of fleas per infested bird:	1·6	1·9	1·5

extreme, we find a rock pipit retrapped up to ten times over a period of 2 months without becoming infested. Another rock pipit was retrapped twelve times during August and was positive on three occasions. One second-brood starling was positive on six consecutive trappings during August acquiring in all eleven specimens of *C. gallinae*. Quite a feature of the retraps is the number of these birds which become infested with more than one specimen, in fact the mean per positive retrap is 1·5. It

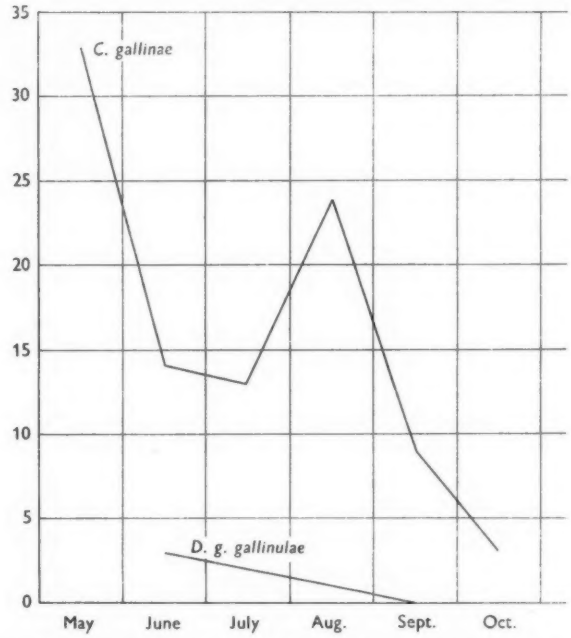


Fig. 9. Starlings: percentage infestation with *C. gallinae* and *D. g. gallinulae*.

Table 15. *Number of fleas per infested starling in June, July and August*

	1952	1953	1954	1955
Starlings with 0 fleas	55	Not known	94	123
Starlings with 1 flea	8	2	11	20
Starlings with 2 fleas	3	0	1	8
Starlings with 3 fleas	1	1	0	4
Starlings with 4 fleas	2	1	0	2
Starlings with 7 fleas	0	0	0	1
Starlings with 8 fleas	1	0	0	0
Starlings with 10 fleas	1	0	0	0
Starlings with 20 fleas	1	0	0	0
Starlings with 22 fleas	1	0	0	0
Number of birds	73	?	106	158
Number of infested starlings	18	4	12	35
Infestation (‰)	24·6	Not known	12·1	22·7
Mean number of fleas per infested bird	4·7	2·2	1·1	1·8

was not unusual to find retrapped juvenile starlings or rock pipits harbouring 3 or 4 fleas at a time. While it is easy to explain multiple infestations on birds which have just left the nest, or on adult birds brooding or feeding young, it is more difficult to explain this curious distribution among the retraps where one would anticipate single rather than multiple infestations, unless the hostless fleas are concentrated in certain areas (see p. 393).

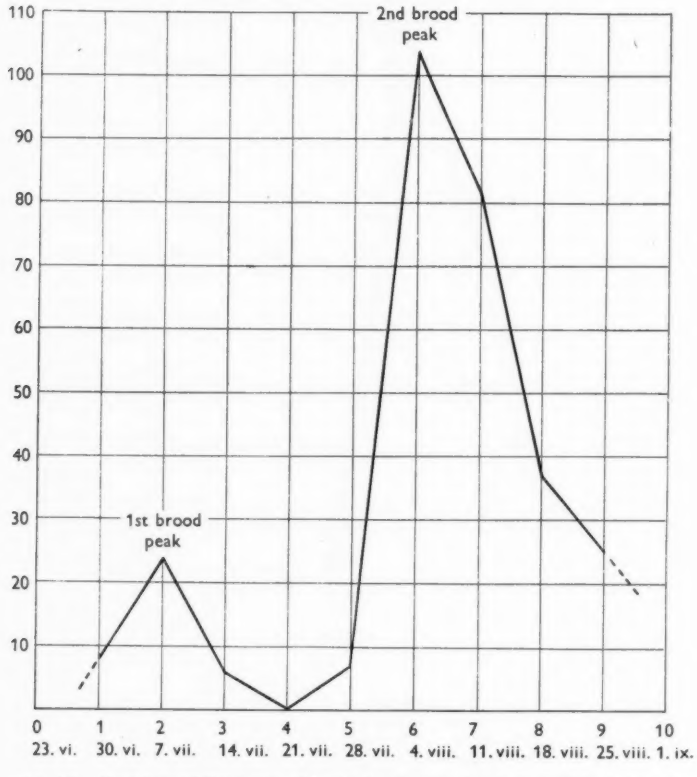


Fig. 10. Number of fleas per 100 starlings examined at weekly intervals (23 June–1 Sept. 1955) by Williamson.

SUMMARY

Approximately 5000 birds (78 species) were trapped on Fair Isle and examined for fleas. Six species of flea were recorded: *D. g. gallinulae* (430 records), *C. gallinae* (205 records), *C. borealis* (126 records), *C. garei* (4 records), *C. fringillae* and *C. vagabundus* (1 record each).

The infestation rate among spring-passage migrants was 52·2 % and among autumn-passage migrants only 2·7 %. Spring migrants released and retrapped after 'de-fleaing' in the Fair Isle apparatus showed a 55 % re-infestation rate. On spring migrants *D. g. gallinulae* was the dominant flea, but on autumn migrants

C. gallinae was dominant. The most heavily infested group of birds were the Turdidae (61 % spring, 3 % autumn infestation rate) and Sylviidae (53 % spring, 1 % autumn infestation rate).

A number of double and triple infestations with 2 or 3 species of flea is recorded. Double infestations involving *D. g. gallinulae* and *C. borealis* occur more frequently than would be expected for purely chance associations. More male birds (90) than female birds (70) were infested.

The sex ratio of the fleas (on the bodies of birds) was: *D. g. gallinulae* 294 males, 325 females; *C. gallinae* 115 males, 214 females; *C. borealis* 77 males, 77 females. On the spring migrants there was an excess of male *D. g. gallinulae* and on the autumn migrants an excess of females.

On the resident birds there was definite evidence of host preference. The starling was the chief host of *C. gallinae*, the pipits of *D. g. gallinulae* and the wheatear of *C. borealis*. The re-infestation rate of 'de-fleaed' retraps showed a higher rate than original trappings in all cases, but the lack of *D. g. gallinulae* on retrapped starlings suggested some host selection on the part of the flea.

The proportions of the three principal species of flea found in (i) the nest of the wheatear, and (ii) on the body of the bird were strikingly different.

My most grateful thanks are due to Kenneth Williamson for making this collection of fleas and for the immense trouble he has taken in sending me information about the hosts and various other points relating to the collection. I am also very grateful to G. Stansfield for his help in checking numerous records and for showing me his figures relating to the 1956 collecting on Fair Isle. I would also like to thank G. H. E. Hopkins and F. G. A. M. Smit for reading the manuscript and making many helpful suggestions, and R. C. Campbell for kindly examining the figures relating to the double and triple infestations.

REFERENCES

Bates, J. K. (1956). Host selection in bird fleas. British Ecological Society Autumn Meeting. *J. Anim. Ecol.* **25**, 475–6.

Butterfield, W. A. (1952). The nesting of Wheatears in 1952. *Fair Isle Bird Obs. Bull.* **8**, 9–10.

Corbet, G. B. (1956). The life-history and host relations of a hippoboscid fly *Ornithomyia fringillina* Curtis. *J. Anim. Ecol.* **25**, 403–20.

Eagle Clarke, W. (1912). *Studies in Bird Migration*. 2. London.

Ioff, I. (1941). The ecology of fleas in connection with their epidemiological importance. (In Russian.) *Pyatigorsk. Ordzhonik. kraev. Izd.* 116 pp., 9 figs., 1 chart.

Jordan, Karl & Rothschild, N. (1910). List of Siphonaptera collected in Portugal. *Novit. Zool.* **8**, 551–4.

Nordberg, Sven (1936). Biologisch-ökologische Untersuchungen über die Vogelnidicolen. *Acta zool. fenn.* **21**, 1–168.

Rothschild, M. (1948). Bird fleas collected by Miss Theresa Clay, Colonel Richard Meinertzhagen and Captain W. H. Pollen in the Island of Ushant, Brittany, France, with a note on the distribution of *Ceratophyllus borealis* by Rothschild (1907). *Entomologist*, **81**, 84–95.

Rothschild, M. (1952). A collection of fleas from the bodies of British birds, with notes on their distribution and host preferences. *Bull. Brit. Mus.* (*Nat. Hist.*) *Ent.* **2**, 185–232.

ROTHSCHILD, M. (1955). The distribution of *Ceratophyllus borealis* Rothschild 1906 and *C. garei* Rothschild 1902, with records of specimens intermediate between the two. *Trans. R. Ent. Soc. Lond.* **107**, 295–317.

ROTHSCHILD, M. & CLAY, T. (1952). *Fleas, Flukes and Cuckoos.* (New Naturalist series.) London: Collins.

SMIT, F. G. A. M. (1955). Two new subspecies of fleas (Siphonaptera) from the British Isles, with a Discussion on their distribution. (Jointly with Miriam Rothschild.) *Trans. R. Ent. Soc. Lond.* **107**, 341–72.

STANSFIELD, G. (1957). The fleas of Skokholm Island. *Ent. Mon. Mag.* **93**, 96–201.

THOMPSON, G. B. (1954). The parasites of British birds and mammals. XXVII. Some notes on *Ceratophyllus borealis* Rothschild (1907). *Ent. Mon. Mag.* **90**, 4–9.

WATERSTON, J. (1910). On some habits and hosts of bird Ceratophylli taken in Scotland in 1909; with description of a new species (*C. rothschildi*) and records of various Siphonaptera. *Proc. R. Phys. Soc. Edinb.* **188**, 73–91.

WILLIAMSON, K. (1952). Migrational drift in Britain in autumn 1951. *Scot. Nat.* **64**, 1–18.

WILLIAMSON, K. (1953). Migration into Britain from the north-west in autumn 1952. *Scot. Nat.* **65**, 65–94.

WILLIAMSON, K. (1954). The Fair Isle apparatus for collecting bird ectoparasites. *Brit. Birds*, **47**, 234–5.

WILLIAMSON, K. (1957). *Fair Isle Bird Obs. Bull.* **3**, 110–60.

(*MS. received for publication* 30. x. 1957.—Ed.)

ON *CERCARIA MEHRAI* FARUQUI, 1930 WITH NOTES ON ITS LIFE-HISTORY

By G. P. JAIN, M.Sc., D.Phil.

Department of Zoology, Mahakoshal Mahavidyala, Jabalpur (M.P.)

(With 4 Figures in the Text)

INTRODUCTION

The object of the present investigation was a more complete description of the structure, and the elucidation of the life cycle, of *Cercaria mehrai* Faruqui, 1930, which was found to infect about 50% of snails, *Indoplanorbis exustus*, in the Allahabad district during the months of July to December.

The livers of infected snails were pale yellow. The first rise in the infection rate occurred in the months of July and October and was followed by a fall in November and December. In December the infection completely disappeared. Further observations indicate that the cercariae emerge from the tissues of their hosts, re-enter *I. exustus* and, after 6–7 hr., encyst in large numbers in the region of the mantle. If they fail to do this, they die and sink to the bottom of the water. The number of cysts varies in different snails from twelve to some hundreds.

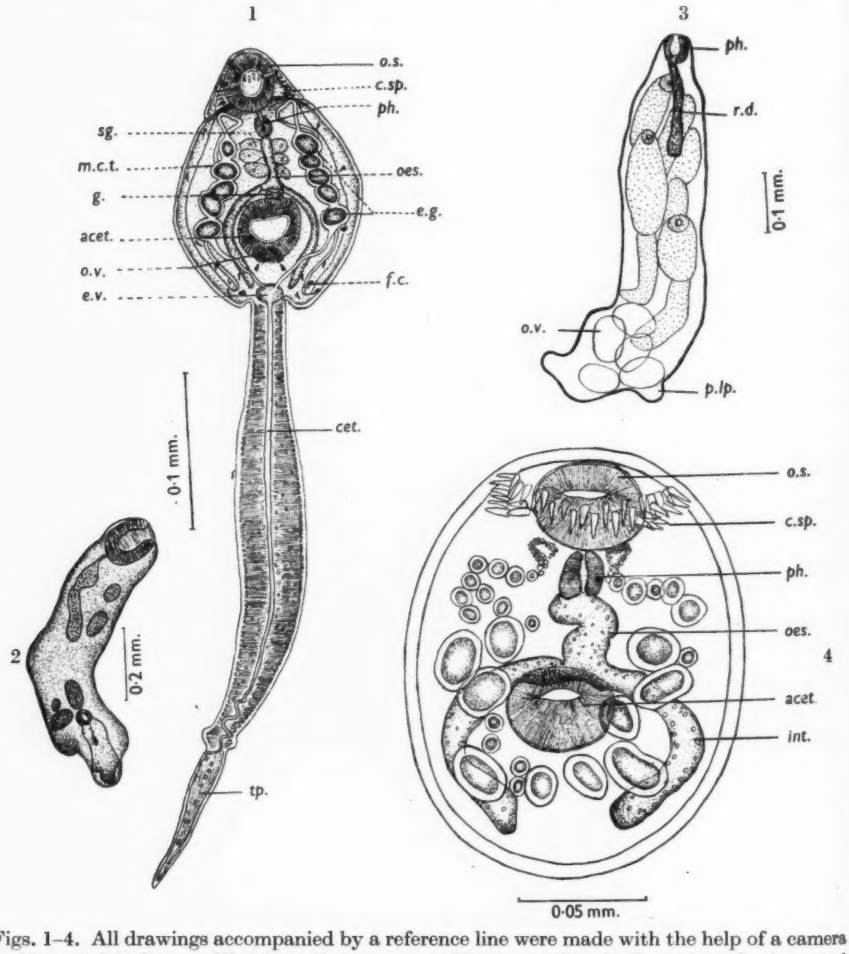
The intra-molluscan phase of the life-cycle of the present form involves the gradual development of the miracidium after its penetration in the molluscan host into a mother redia. The mother rediae were observed in the pre-oesophageal region of the snail, where they produce large numbers of daughter rediae by multiplication of germ cells (primary polyembryony) and by a special form of polyembryony involving germ masses (secondary polyembryony). The daughter rediae fix themselves in the liver mass, undergo further development and produce large numbers of cercariae. The cercariae are also produced by multiplication of germ cells. The development of cercariae was not studied, but their escape from daughter rediae was noticed on several occasions. The birth pore is visible only when a cercaria is in the act of emerging from the redia. The entire process of development, from the entry of the miracidium until the cercariae are matured, takes about 8 weeks.

Cercaria mehrai Faruqui, 1930

The cercaria (Fig. 1) is small. It swims actively at all depths in water. While it is swimming, it contracts its body and becomes spherical and the tail lashes vigorously, propelling the cercaria through the water. The cercaria also moves rapidly on a substratum by the alternate use of its two suckers.

The body, which is capable of considerable extension and contraction, measures, when it is fully extended, 0·327 mm. long and 0·186 mm. broad. The tail is 0·352 mm. long. The greatest width of the body is at the level of the acetabulum. Anteriorly the body becomes somewhat rounded in outline and has, on its ventral side, a distinct collar. The collar is armed with forty-three spines arranged in two

rows. The posterior region of the body is not exactly oval but is slightly elongated. The whole surface of the body is covered with spines directed backwards and arranged in transverse rows. Beneath the cuticle are numerous rectangular cystogenous cells filled with parallel protoplasmic rods. The anterior region is occupied by a well-developed oral sucker, which has a diameter of 0·027 mm. The acetabulum is situated at about 0·024 mm. of the length of the body from the posterior end. The long tail has no fin fold and is attached to the posterior end of the body. At



Figs. 1–4. All drawings accompanied by a reference line were made with the help of a camera lucida and details were filled in at the same magnification. Fig. 1. *Cercaria mehrai*, ventral view. Fig. 2. Mother redia showing an immature daughter redia in the body. Fig. 3. Daughter redia showing immature cercariae and germ cells. Fig. 4. Cyst of *C. mehrai*, ventral view. *Abbreviations*: *acet.*, Acetabulum; *c.sp.*, collar spine; *cet.*, caudal excretory tubule; *e.v.*, excretory vesicle; *e.g.*, excretory granule; *f.c.*, flame cell; *g.*, genital atrium; *int.*, intestinal caeca; *m.c.t.*, main collecting tubule; *o.s.*, oral sucker; *oes.*, oesophagus; *o.v.* ovary; *ph.*, pharynx; *p.lp.*, posterior locomotor process; *r.d.*, rhabdocoel gut; *s.g.*, salivary gland; *tp.*, tapering cone.

its free end it has a conical portion, 0·089 mm. long, which is distinctly separated from the tail stem. At the junction of the terminal cone with the tail proper there are characteristic bulb-like processes. The tapering cone of the tail is quite flexible and is covered with thin cuticle devoid of transverse muscular striations.

The subterminal mouth leads into the cavity of the oral sucker. The prepharynx is narrow and well developed and extends from the oral sucker to the pharynx. The oesophagus is long and much thicker than the prepharynx and it bifurcates in front of the anterior margin of the acetabulum into two intestinal caeca. The caeca pass round the acetabulum and back to the posterior end of the body. The salivary glands, six in number, occupy the central, pre-acetabular region on each side of the oesophagus. Each cell of these glands contains a prominent nucleus.

The excretory bladder is spherical and is situated at the extreme posterior end of the body. The two main collecting ducts originate anteriorly by a simple aperture from the dorsal surface of the excretory bladder. The excretory bladder may assume various shapes, due to contraction and expansion of the body. The main excretory canals arise from the anterior aspects of the bladder and pass forwards and outwards. Their posterior ends are thin-walled, of very narrow calibre and difficult to see. These main tubes become, in front of the middle of the acetabulum, much broader and they are filled with five globular or oval refractile masses of excretory material, which make them very prominent. This broad part of the duct passes a little forward and then becomes, at the level of the pharynx, narrower and proceeds forwards as a narrow duct, which runs first outwards and then inwards and backwards to form a loop. From this point the ducts then continue their backward course towards the hinder end of the body, where they turn again towards the anterior end of the body. As the backward course of these ducts lies mostly close against, and parallel to, the anteriorly directed main excretory ducts, it is difficult to detect them.

Twelve pairs of flame cells were observed in the body, but no flame cells are present in the tail proper. Five pairs of flame cells are pre-acetabular. The first pair lies close against the margin of the oral sucker in the lateral region; the second pair lies just anterior to the loop of the main excretory duct; the third pair is situated posterior to the loop near the first excretory granule; the fourth pair is situated laterally at the level of the third excretory granule; the fifth pair is just anterior to the acetabulum in the space enclosed by the bifurcation of the intestinal caeca; the sixth pair lies at the level of the acetabulum, just posterior to the fifth excretory granule; the seventh pair lies just posterior to the acetabulum; and the remaining five pairs lie posteriorly near, and outside, the posterior region of the intestinal caeca. The caudal excretory canal originates from the posterior end of the excretory bladder, on its dorsal side, and runs back through the tail stem. This canal varies much in calibre and extends back up to the point where the tail gives rise to the tapering cone. The caudal excretory canal runs straight for most of its course, but in the posterior region it becomes somewhat wavy for a short distance. It then turns laterally to open, below the bulbous processes of the tail, through a minute excretory pore to the exterior. No lateral canals are given off from the caudal excretory duct.

The primordium of the genital organs is represented by a mass of small rounded cells situated at the posterior extremity of the acetabulum, in front of the excretory bladder. Another mass of cells lies in the space enclosed by the intestinal caeca in front of the anterior margin of the acetabulum. The former mass probably represents the rudiments of the ovary and uterus and the second mass represents, according to Sewell, the genital pore.

The redial generation

Mother rediae

The mother rediae (Fig. 2) are large and have one very distinct feature; the intestine is very small. The snail which contained this form harboured four or five mother rediae. They contain no free daughter rediae, and, even in the largest mother rediae, none of the embryos appeared ready to escape. The smallest mother rediae measured 0·467 by 0·154 mm. and the largest 1·116 by 0·279 mm. The pharynx is characteristically larger than that of the largest daughter rediae. These mother rediae have germinal masses with both unicellular and multicellular components attached at the posterior end of the body cavity. The daughter rediae were counted in nine of the snails and their numbers vary from 187 to 456, the average number being 380. These counts are all somewhat below the true numbers, because it is easy when infections are as heavy as these were to miss the smallest immature rediae.

Daughter rediae

The smallest daughter rediae (Fig. 3), in which no cercariae have developed, are colourless and measure 0·64 mm. long and 0·112 mm. broad. The width gradually increases from the bluntly rounded anterior end to a point in front of the posterior locomotor appendages. The mouth is terminally situated at the anterior end and leads back into a well-developed muscular pharynx. The pharynx is followed by a short narrow oesophagus, which soon widens out into a well-developed intestine, which extends backwards to a point 0·192 mm. from the anterior end of the body.

In the more mature, but not yet fully grown, stage, the posterior locomotor appendages are prominent and the whole space of the body is filled with a large number of germ cells.

The daughter rediae do not show much difference in their contracted or expanded conditions, and a single change that they show is due to the eversion of the pharynx. They measure 1·312–2·4 mm. in length and 0·24 mm. in breadth. The mouth is situated at the anterior end and leads back into a well-developed pharynx, which is protrusible and measures 0·224 mm. in diameter. Behind the pharynx there is a short, comparatively narrow portion of the gut, brown in colour, which extends backwards to a point 0·24 mm. from the pharynx. The brown colour of the gut is due to the food particles in it. The collar is present, but is not clearly visible. Behind the collar there is a depression in the body which contains the birth pore. The body of the redia is provided with a pair of locomotor processes in the hinder part which help in its locomotion. Each redia contains a large number of cercariae

in various stages of development. Some of these are fully developed and can be seen moving inside the body cavity. They emerge out of the birth pore by their anterior or posterior ends.

The excretory system of the older rediae was so heavily pigmented that it could not be studied properly and in the young rediae the flame cells are so small that it is difficult to trace them. The germ balls occupy the extreme posterior end of the body.

Metacercaria

The cysts of *Cercaria mehrai* (Fig. 4) are almost spherical being 0·143 by 0·104 mm. The cyst wall is 0·0068 mm. thick. It is transparent and composed of two layers. The young distome lies contracted in the cyst, occupying almost the whole of the space within the cyst wall. The anterior end of the mature cercaria is rounded and has an oral sucker, which measures 0·0374–0·0459 mm. in diameter. The sucker is surrounded by the collar, which is armed with forty-three collar spines, arranged in double rows. Behind the collar the body is covered with numerous inconspicuous spines arranged in transverse rows. The acetabulum is situated at about one-third of the body length from the posterior end and is 0·051 by 0·0578 mm.

The mouth is subterminal and leads back into the cavity of the oral sucker, behind which is a prepharynx. The pharynx, 0·017 mm. in diameter, is muscular and is followed by the oesophagus, which is 0·0306 mm. long. In front of the acetabulum the oesophagus divides into two intestinal caeca, which are continued almost to the posterior end of the body. These caeca are tubular and very prominent and in the lumen of each of them there are large numbers of small, globular, refractile bodies.

The excretory system in the metacercaria is very similar to that of the cercaria. The excretory loop is likewise filled with numerous small excretory granules. The genital organs are represented by two small masses of cells, one on the anterior side and the other on the posterior side of the ventral sucker.

DISCUSSION

C. mehrai Faruqui, 1930 and *C. palustris* Chatterji, 1933 are so closely related to each other that they exhibit no specific characters by which they can be distinguished. Faruqui (1930) found it practically impossible to see any collar spines in his form and he described it as spineless echinostome cercaria, whereas Chatterji (1933) omitted the exact number of the spines of *C. palustris* and also their mode of distribution.

The study of these forms has convinced the author that they are characterized by the presence of forty-three collar spines arranged in two rows uninterrupted dorsally. A similar arrangement of collar spines is present in their adults *Paryphostomum mehrai*. The adults were obtained by feeding the cysts of *Cercaria mehrai* to white rats, all of which were known to be free from trematode infection. The metacercariae emerged in the small intestine of the hosts and matured within a period of 20 days. The adults thus obtained were identified as *Paryphostomum*

mehrai. The body of the parasite has an arch-shaped collar at the anterior extremity. The collar is armed with forty-three collar spines arranged in two rows uninterrupted dorsally. The spines in the terminal group are a little larger than those in the marginal groups.

The body beneath the cuticle is crowded heavily with cystogenous cells, which contain rod-like granules. The intestinal caeca bifurcate just in front of the acetabulum and extend up to the posterior end of the body. There are present, lateral to the oesophagus, three pairs of salivary glands. The most striking similarity between *Cercaria mehrai* and *C. palustris* is that the tail ends abruptly in a tapering cone, which is very contractile. The excretory system is similar in both the forms. It resembles that of *Echinostoma recurvatum* (Mathias, 1925), which has forty-five spines.

As regards the systematic position of *Cercaria mehrai*, Chatterji was justified in placing his form, *C. palustris*, in the '*Eschinata* group' of Sewell (1922). Faruqui (1930) wrongly placed his form, *C. mehrai*, in the '*Agilis* group' of Sewell. Sewell considered that the excretory system is one of the characters which separate echinostome cercariae into three groups, namely, '*Echinotoides*' '*Coronata*' and '*Echinata*' groups. *Cercaria mehrai* comes much closer to the *Echinata* group, because the spines are arranged in two rows, there is no fin fold of the tail, the course of the excretory canal is identical and the cercariae encyst in the same molluscan host.

The writer wishes to express appreciation for suggestions and advice from Professor Paul C. Beaver and to acknowledge the assistance generously given by Professor H. R. Mehra. The work was carried out under the terms of the Uttar Pradesh Government Research Grant to the University of Allahabad.

REFERENCES

CHATTERJI, R. C. (1933). On an Echinostome cercaria, *Cercaria palustris*, with notes on its life-history. *Bull. Acad. Sci. Univ. Prov.* **2**, 193–201.

FARUQUI, A. J. (1930). On a new species of *Cercariae indicae* found in *Indoplanorbis exustus* (Deshayes). *Indian J. med. Res.* **17** 1205–14.

MATHIAS, P. (1925). Recherches expérimentales sur la cycle évolutif de quelques trématodes. *Bull. Biol.* **59**, 1–123.

(*MS. received for publication* 16. XI. 1957.—Ed.)

THE VECTOR OF *HEPATOZOON SCIURI*

By B. DASGUPTA and KAMANI MEEDENIYA

Department of Parasitology, London School of Hygiene and Tropical Medicine

(With 7 Figures in the Text)

I. INTRODUCTION

The occurrence of the gametocytes of *Hepatozoon sciuri* in the blood of the squirrels of England* was first reported by Coles (1914), who described this parasite as *Haemogregarina sciuri*. Herman & Price (1955) reported the occurrence of the same parasite in the grey squirrels (*Sciurus carolinensis*) of Washington D.C. and Maryland areas of the U.S.A.

Our attention was drawn by Mrs M. Vizoso of the Infestation Control Division, Ministry of Agriculture, Fisheries and Food, to the fact that a large number of squirrels (*S. carolinensis* and also *S. vulgaris*), trapped by her in various parts of England, were found to harbour *Hepatozoon sciuri* in the leucocytes. The grey squirrels (*Sciurus carolinensis*) were heavily infested with a flea which was identified by the British Museum of Natural History as *Orchopeas wickhami*. These fleas were also found on the red squirrels (*Sciurus vulgaris*) on various occasions. The heavy infestation by these fleas of squirrels which were infected with *Hepatozoon sciuri* aroused the suspicion that these insects might be acting as the vector of this infection, and we accordingly undertook an investigation of this problem.

II. MATERIAL AND METHODS

Fleas collected from the infected squirrels at Alice Holt, Surrey, were kindly made available to us by Mrs M. Vizoso. Fleas were also collected by one of us (K.M.) from the nests of wild squirrels at various places in Essex and Surrey, and Dr J. F. D. Frazer, of the Charing Cross Hospital, kindly sent us some squirrels, carrying fleas, shot by him in Kent.

The abdomen of each flea was fixed in Carnoy's fluid for 2 hr. They were dehydrated in dioxan, infiltrated with 2% solution of celloidin in methyl benzoate, cleared in benzene, embedded in paraffin at 60° C., and cut 5–6μ thick.

We examined these fleas in serial sections. In the course of this examination we came across sporogony stages of *Hepatozoon*, which are described in this communication. We have already demonstrated some sections of these infected fleas at the Laboratory Meeting of the Royal Society of Tropical Medicine and Hygiene (Dasgupta & Meedeniya, 1958).

In addition, a number of 'wild' fleas which had fed on a squirrel showing *Hepatozoon* infection in the leucocytes were fixed in batches at an interval of 24 hr., up to the 11th day after the blood meal, and examined in serial sections.

* Species not stated.

III. SPOROGONY OF *HEPATOZOON*

The results of this investigation were at first disappointing. Successive batches of fleas collected from the infected squirrels which were in captivity, and also those collected from the nests of wild squirrels were found to be free of *Hepatozoon* infection. We were about to give up this search when, in one batch of fleas collected from the squirrels sent by Dr Frazer, three specimens were found to be infected by *Hepatozoon*. One of these three specimens had a slight infection, while the other two were very heavily infected. In the heavily infected specimens there was no trace of fat-cells, and the parasites filled up the entire space between the alimentary canal and the body wall.

The earliest stage of the parasite observed was the round multinucleate zygote (Fig. 1), which measured $35{\cdot}8 \times 35{\cdot}8\mu$. The nuclei stained deeply with Ehrlich's haematoxylin, but were negative to Feulgen's technique.

The next stage observed was that of early sporoblast formation (Figs. 2, 3) measuring $99{\cdot}3 \times 99{\cdot}3\mu$ and $99{\cdot}3 \times 38{\cdot}6\mu$ in two separate instances. The large parasitic body was characterized by a central residual mass, on the periphery of which small projections of vacuolated cytoplasm, each with a nucleus, remained attached. The nuclei at this stage were Feulgen-positive. Bright red granules were found arranged around the nuclear membrane, and one or more such granules lay inside the nucleoplasm.

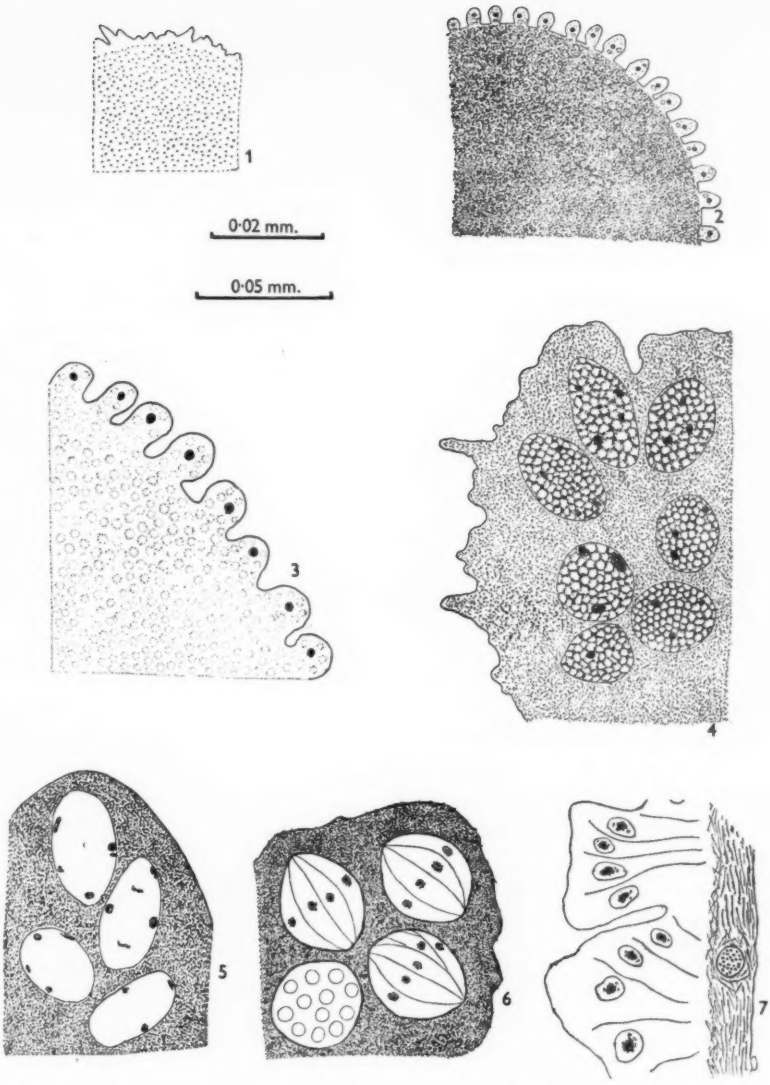
The next stage encountered was that of large sporoblasts, measuring about $22 \times 8{\cdot}2\mu$ and lying inside the residual mass of the parasitic body (Figs. 4, 5). The nuclei of the sporoblasts were strongly Feulgen-positive, the distribution of the Fuelgen-positive material in the resting nuclei being similar to that seen in the preceding stage. The mature sporocysts (Fig. 6), which were situated in the residual mass, contained twelve sporozoites, which measured $17{\cdot}6 \times 3{\cdot}5\mu$ approximately. The nucleus of the sporozoite was strongly Feulgen-positive, the distribution of the Feulgen-positive material being similar to that of the resting nuclei of the sporoblasts.

An attempt was made to establish a laboratory infection in the fleas by feeding them on a squirrel which showed *Hepatozoon* infection in the leucocytes. These fleas were examined in batches up to the 11th day after the blood meal. In serial sections no trace of *Hepatozoon* infection could be seen, but a very heavy infection of the gut wall by a small intracellular parasite was noticed. It may be mentioned that a similar intracellular parasite was previously encountered in one of the earlier specimens of fleas (Fig. 7). We wish to reserve our comment on the identity of this intracellular parasite at this stage. It may also be mentioned here that *Hepatozoon* and a Schizogregarine infection* occurred side by side in the same flea.

IV. DISCUSSION

The occurrence in nature of the sporogony stages of *Hepatozoon* in the fleas strongly suggests that these insects act as vectors of this parasite. It is, however, not clear why a large number of fleas collected from infected squirrels did not

* A description of this Schizogregarine is in the press.



Figs. 1–7. Camera lucida drawings of the sporogony stages of the parasite. (Scale 0·05 refers to Fig. 2, and scale 0·02 to the rest of the figures.)

Fig. 1. Part of a section of the early multinucleate zygote: small dot-like nuclei stained well with Ehrlich's haematoxylin, but were negative to Feulgen's technique.

Fig. 2. Part of a section of the parasite at the early stage of sporoblast formation. Small outgrowths of cytoplasm are produced at the periphery of the large parasitic body; the central area of the parasite is strongly basophilic, and the nuclei in the outgrowths at the periphery are Feulgen-positive.

Fig. 3. Magnified view of the same stage as shown in Fig. 2.

Fig. 4. A number of sporoblasts embedded in residual mass of the oocyst: the nuclei are at various stages of division; the cytoplasm is vacuolated.

Fig. 5. Same stage as in Fig. 4: the nuclei are positive to Feulgen's nucleal reaction and the residual mass is deeply stained with Alcian blue.

Fig. 6. Mature sporozoites in the sporocysts: nuclei are Feulgen-positive; the residual mass is deeply stained with Alcian blue; transverse section through one of the sporocysts reveals the presence of twelve sporozoites in it.

Fig. 7. Section through the stomach wall of a flea showing the presence of an unidentified intracellular parasite.

reveal any infection at all. It is also not possible at this stage to offer an explanation as to why the attempt to establish a laboratory infection in the fleas did not succeed. Clark (1955) found that the flea *Orchopeas howardii*, an ectoparasite of the grey squirrels (*Sciurus carolinensis*) of the U.S.A., was not infected with *Hepatozoon sciuri*. The supposed developmental stages of this parasite were found to occur in the mites *Haemogamasus barberi* and *Echinolaelaps echidninus*. The latter species of mite could be easily cultured in the laboratory, and was successfully infected with *Hepatozoon*, which reached the infective sporozoite stage in the body cavity of *Echinolaelaps echidninus* in approximately 30 days. Similar forms were commonly found in sections of naturally infected *Haemogamasus barberi* taken from the squirrel nest-boxes in the field. The same author has kindly informed us (Clark; personal communication 1957) that the stages of *Hepatozoon* observed by him in these mites are now considered to belong to a new species, which is designated by him as *Hepatozoon griseisciuri* sp.nov., a description of which is in the press.

Although the fact that *Hepatozoon* can develop in the fleas has been demonstrated by us beyond reasonable doubt, we do not exclude the possibility that some other arthropod may also act as the vector of *H. sciuri*.

V. SUMMARY

A description is given of the stages of sporogony of *Hepatozoon sciuri*, which have been found in three wild fleas (*Orchopeas wickhami*) out of a total of nearly sixty specimens collected from squirrels in England. Although an attempt to infect the fleas in the laboratory, by feeding them on infected squirrels, failed, the above findings indicate that these fleas may act as vectors of the squirrel parasite.

We wish to record our thanks to Professor P. C. C. Garnham for his kind interest and constructive suggestions regarding this work. We are specially indebted to Mrs M. Vizoso and to Dr J. F. D. Frazer for sending us fleas and squirrels from time to time. Our thanks are due to the authorities of the British Museum of Natural History for the identification of the flea.

REFERENCES

CLARK, G. M. (1955). The development of *Hepatozoon sciuris*, Coles 1914, in two parasitic Laelaptid mites. *J. Parasitol.* **41**, 6, Sect. 2, Suppl., 17.

COLES, A. C. (1914). Blood parasites found in mammals, birds and fishes in England. *Parasitology*, **7**, 17.

DASGUPTA, B. & MEEDENIYA, K. (1958). Sporogony stages of *Hepatozoon sciuri* (Demonstration). *Trans. Roy. Soc. Trop. Med. & Hyg.* **52**, 10.

HERMAN, C. M. & PRICE, D. L. (1955). The occurrence of *Hepatozoon* in the grey squirrel (*Sciurus carolinensis*). *J. Protozool.* **2**, 48.

(*MS. received for publication* 4. XII. 1957.—Ed.)

FACTORS AFFECTING THE GROWTH AND BEHAVIOUR OF AXENIC STRAINS OF *ENTAMOEBA INVADENS* RODHAIN, 1934

By ELSPETH W. McCONNACHIE*
Molteno Institute, University of Cambridge

CONTENTS

		PAGE
I.	Introduction	423
II.	Material and methods	423
III.	Characteristics of axenic strains of *Entamoeba invadens* growing in 0·75% NaCl + rat liver	424
IV.	Physico-chemical factors affecting the growth of axenic strains of *Entamoeba invadens* in saline-liver medium	426
V.	Tissue factors affecting the growth of axenic strains of *Entamoeba invadens*	427
VI.	Discussion	430
VII.	Summary	431
	References	432

I. INTRODUCTION

Bacteria-free cultures of *Entamoeba* were first obtained by Lamy (1948*a*, *b*) and Miller (1951, 1953), working independently, who prepared cultures of *Entamoeba invadens* in media containing chick embryo or liver. Although neither worker states whether living embryo or liver cells are an essential part of his medium, these media may be regarded as axenic, in the strict sense of the word (Dougherty, 1953), as the embryo and liver tissue on which they are based are not living in the sense that they comprise multiplying cells. In addition, Miller (1953) obtained growth of amoebae in a medium containing only the supernatant from a homogenized, centrifuged preparation of liver tissue.

Several years have passed since these axenic strains of *E. invadens* were first obtained, but little information is available on their growth and behaviour *in vitro*. The present account describes some of the characteristics and factors affecting the growth of axenic strains of *E. invadens* growing in Miller's saline-liver medium.

II. MATERIAL AND METHODS

Two axenic strains of *E. invadens* were used. Strain N originates from a strain of *E. invadens* isolated by the late Professor J. Rodhain of Antwerp, and its preparation has already been described (McConnachie, 1956); this strain has now been maintained in axenic culture, without antibiotics, for more than 3½ years, during which its growth characteristics have remained unchanged. Strain BN, derived from a strain of *E. invadens* (BAH) isolated by Dr R. A. Neal, was prepared in a similar way to strain N. The mixed, unknown flora of strain BAH was first simplified by treating cysts growing in diphasic horse-serum–Ringer-eggwhite, containing

* Member of the Scientific Staff of the Medical Research Council.

starch (HSre + S of Dobell & Laidlaw, 1926) with potassium permanganate and mercuric chloride (McConnachie, 1956). Amoebae obtained from cultures derived from these treated cysts were then inoculated into saline-liver medium containing penicillin and streptomycin, as in the preparation of strain N. The resulting strain, designated BN, was maintained for twelve subcultures in medium containing antibiotics, over a period of 23 weeks; subculture into saline-liver medium without antibiotics, and subsequent microscopic examination and subculture into Robertson's meat broth and glucose broth, proved that strain BN was bacteriologically sterile. The strain has now been maintained in saline-liver medium for more than one year.

The majority of the observations have been made on strain N, and these have been confirmed on strain BN under similar conditions. Strain N was used in all the experimental work.

Stock cultures of the axenic strains are maintained at 24° C. in test-tubes containing 10 ml. of sterile 0·75 % NaCl and a slice of sterile, untreated rat liver, the tubes being sealed, after inoculation, with a layer of sterile vaseline (McConnachie, 1956). Subcultures are made at intervals of approximately 3 weeks by pipetting a small amount of the sediment from the bottom of a culture containing amoebae into a new tube of culture medium.

The axenic cultures of *E. invadens* are examined regularly for sterility; Gram-stained smears of the stock cultures, subinoculations into Robertson's meat broth and glucose broth, and direct microscopic examination have never revealed the presence of bacteria or fungi.

The stock medium consisted of 0·75 % NaCl containing rat liver; amoebae growing in cultures of this medium were used for the inocula in experiments to test the growth-promoting properties of other preparations. Unless otherwise stated, both test and stock media were sealed with vaseline after inoculation, and incubated at 24° C. for 3–4 weeks. Starch was added to those test media which, it was considered, might be deficient in carbohydrate. In each experiment, the viability of the inoculum was confirmed by inoculating amoebae into a tube of stock medium; growth in this control culture provided the standard for comparison with growth in the test cultures.

III. CHARACTERISTICS OF AXENIC STRAINS OF *ENTAMOEBA INVADENS* GROWING IN 0·75 % NaCl + RAT LIVER

Even after three and a half years in axenic culture, the growth of strain N remains unchanged, and there has been no marked improvement or reduction in the numbers of amoebae produced, nor in their rate of growth. Growth of the axenic strains of *E. invadens* is very poor, compared with that of *E. invadens* in cultures accompanied by bacteria. Owing to the small numbers of amoebae produced, it was not feasible to construct growth curves for these axenic cultures; a rough comparison of the intensities of growth in axenic and monobacterial cultures was obtained, however, by counting the number of amoebae observed in similar microscopic fields under the same magnification (Table 1); in each strain, the samples of amoebae were taken from cultures showing approximately maximal growth.

As found by Miller (1953), cultures of axenic amoebae remain viable for considerable periods. Maximum growth, as determined by observation, is reached within approximately 3 weeks, after which the numbers of amoebae decrease gradually over the subsequent 2–4 weeks. Cultures usually remain viable, at 24° C., up to approximately 10 weeks; at this temperature, it is unusual to obtain living amoebae from cultures of more than 12 weeks, although one culture was still viable, as determined by subculture, after 23 weeks' incubation. The longevity of the cultures is increased by incubation at room temperature (16–20° C.), and several cultures have remained viable at these lower temperatures for up to 20–25 weeks.

Table 1. *Comparative yields of amoebae in axenic and monobacterial cultures*

Strain	Medium	Age of culture (weeks)	No. of amoebae per low-power field* (objective 10×, ocular 9×)
N	Saline + rat liver	3	1·6
N	Saline + rat liver	3	2·1
N	Saline + rat liver	3	6·6
N	Saline + rat liver	3	7·1
N	Saline + rat liver	6	2·4
N	Saline + rat liver	7	2·1
BC	eysaph + S	3	29·3
BC	eysaph + S	3	33·1
BC	HSre + S	3	65·1

* Mean of 10 fields.

Strain BC: monobacterial strain of *E. invadens* and *Esch. coli*; S: starch; eysaph: egg-yolk infusion (Balamuth & Sandza, 1944); HSre: horse-serum—Ringer-eggwhite (Dobell & Laidlaw, 1926).

It was previously found (McConnachie, 1956) that cyst formation in the axenic strain N was comparatively rare, but the strain has not lost its ability to produce cysts after prolonged axenic cultivation. Careful examination of cultures growing in saline-liver medium frequently fails to reveal the presence of any cysts, but small numbers of cysts have been found in some cultures in which there is a good growth of amoebae. These cysts are always isolated, and are usually found amongst portions of disintegrating liver tissue; mass encystation, with the formation of large clumps of cysts, as occurs in certain mono- or polybacterial strains of *E. invadens*, has never been observed in the axenic strains. Cysts formed in axenic cultures appear to mature normally; four-nucleate cysts, with and without chromatoids, have been observed.

Several attempts have been made to determine whether cysts formed in axenic cultures are viable. Cultures containing cysts were treated with hydrochloric acid to kill the amoebae (McConnachie, 1955). The sediment from acid-treated cultures was washed in Ringer's solution and inoculated into HSre + S seeded with *Escherichia coli*, but cultures of amoebae have never been obtained in this way. These negative results, however, cannot be regarded as proof of the non-viability of the cysts in the axenic cultures, as experience with cysts produced in mono- and

polybacterial strains of *E. invadens* has shown that their mortality rate is high when inoculated into culture medium. The number of cysts in the inocula prepared from acid-treated cultures was probably not large enough to ensure the survival of even a single cyst with which to initiate a culture of amoebae.

Cyst production is no higher in newly isolated than in long established axenic strains of *E. invadens*. Cultures of strain BN were examined regularly for cysts from the time when the bacteriological sterility of the strain was first established. Cyst production was as sparse and spasmodic in the newly isolated axenic cultures as in those of strain N which has been maintained in axenic culture for $3\frac{1}{2}$ years. Studies on the factors affecting encystation in dibacterial strains of *E. invadens* have shown that the production of large numbers of cysts is correlated with conditions favouring amoebic growth (McConnachie, 1955), and it is probable that encystation in axenic cultures would be stimulated if a medium producing a heavier growth of amoebae could be found.

The marked vacuolation of the amoebae in these axenic cultures of *E. invadens* has already been noted, as has the slightly larger size of the amoebae, compared with that of amoebae in poly- or monobacterial cultures (McConnachie, 1956). The structure of the nucleus, as shown in films fixed in Bouin's solution and stained with iron haematoxylin, is normal in every respect, and multinucleate amoebae do not appear to be more numerous in the axenic than in mono- or polybacterial strains.

Glycogen is present in the cytoplasm of the axenic amoebae, as shown by staining with iodine, and ingested particles of liver are also frequently observed. The slice of liver tissue in the culture tube disintegrates slightly, particularly in tubes of older medium, and the particles of liver ingested are probably derived from these portions of disintegrating tissue. No evidence has been obtained, from the examination of fixed and stained sections of liver removed from cultures, that the amoebae actively penetrate into the liver in the culture medium.

IV. PHYSICO-CHEMICAL FACTORS AFFECTING THE GROWTH OF AXENIC STRAINS OF *ENTAMOEBA INVADENS* IN SALINE-LIVER MEDIUM

Salinity and ionic balance. The optimum concentration of NaCl in which *E. invadens* grows in cultures of saline-liver medium is 0·75–1·0%. No growth occurred in medium containing 0·1, 0·25 or 2·0% NaCl; slight growth occurred when 0·5% NaCl was used. The amoebae were equally vacuolated in all concentrations of saline at which growth occurred.

The addition of K, Ca, Mg, $Fe(NH_4)_2$, SO_4, PO_4 or HCO_3 ions had no effect on the vacuolation of the amoebae, nor on their growth. Both were unaffected when 0·75% NaCl was replaced with Ringer's solution (modified according to Dobell & Laidlaw, 1926), Tyrode's solution or a balanced salt solution used for axenic cultures of *Paramecium aurelia* (van Wagtendonk, Miller & Conner, 1952).

pH. The pH of fresh, unused saline-liver medium ranged from 6·4 to 6·1 (mean 6·2); the pH of 3-week-old cultures of amoebae in this medium ranged from 6·7 to 6·0, with a mean value of 6·5.

The effect of pH on the growth of the axenic strain of *E. invadens* was studied

in a series of experiments in which M/15 phosphate buffers in 0·75% NaCl were substituted for the 0·75% NaCl in the medium (Table 2). No growth occurred below pH 5·0, or above 7·0, although a few amoebae survived at the higher pH. Optimum growth occurred between pH 5·7 and 6·1, and it was noticed that in cultures with higher pH values (just below 7·0), at which growth was poor, the amoebae were less vacuolated than in those with a pH of approximately 6·0.

Table 2. *The effect of* pH *on the growth of an axenic strain of* Entamoeba invadens *in phosphate-saline + rat liver*

pH of medium		Incubation period (weeks)		
A	B	3	4	6
4·8	5·2	−	0	−
4·8	5·3	−	0	−
5·7	5·9	—	+ + +	−
5·7	6·1	+ + + +	−	(+)
6·1	6·1	+ + +	−	−
6·2	6·2	+ +	−	(+)
6·2	6·3	+ +	−	+ +
6·9	6·7	+ +	−	+ +
6·9	6·7	−	0	−
7·3	7·1	0	−	(+)
7·9	7·4	−	0	−
7·9	7·6	(+)	−	0

A: pH before inoculation; B: pH at end of test; (+): very few amoebae; + + + +: amoebae numerous.

Temperature. Axenic cultures of *E. invadens* can be maintained indefinitely at room temperature (16–20° C.), 24° C. or 30° C.; optimum growth occurs at 24° C. No growth occurs at 37° C., at which temperature the amoebae rapidly degenerate

Anaerobiosis. Like all species of *Entamoeba*, *E. invadens* grows only under anaerobic conditions (McConnachie, 1955). The presence of liver tissue in the medium used for the axenic cultivation of *E. invadens* produced a sufficient degree of anaerobiosis for amoebic growth; growth in unsealed cultures, however, was variable, and it was found most satisfactory to seal the cultures, after inoculation, with vaseline, as recommended by Miller.

The intensity of growth in unsealed cultures incubated in an anaerobic jar containing nitrogen was the same as that in cultures sealed with vaseline and incubated in air.

V. TISSUE FACTORS AFFECTING THE GROWTH OF AXENIC STRAINS OF *ENTAMOEBA INVADENS*

Source of liver. Miller (1953) found that liver from a variety of sources supported good growth of axenic strains of *E. invadens*. Strain N of *E. invadens* also grew equally well in saline containing liver from rats, chicks or hamsters, and the vacuolation of the amoebae was not affected by the source of the liver. No difference was found in the growth of the amoebae in medium containing liver from normal male or female rats, or from pregnant rats; nor, as previously

reported, was growth affected by the use of liver from rats which had recovered from an infection with *Trypanosoma lewisi* (McConnachie, 1956).

Age of saline-liver medium. Stock saline-liver medium retains its growth-promoting properties unaltered for up to 10 weeks, when stored in the refrigerator at 8° C. Cultures of amoebae have sometimes grown satisfactorily when inoculated into saline-liver medium which had been stored for 12 weeks at 8° C., and in which the liver often showed considerable signs of disintegration, but such medium does not invariably support growth, and growth has never occurred in medium stored for more than 20 weeks.

Preparations of liver. Miller's finding that a suspension of liver in saline retained the growth-promoting properties of the intact slice of liver was confirmed. Rat liver was passed through a tissue mincer, and suspended in 0·75 % NaCl (approximately 1 g. of liver to 10 ml. of saline), all operations being performed under aseptic conditions. The suspension was added, in 1 ml. volumes, to a series of tubes, each containing 10 ml. of sterile 0·75 % NaCl, and the medium immediately sealed with vaseline. Growth of *E. invadens* in this liver suspension was equal to that in the stock saline-liver medium.

Miller reported that the supernatant from a liver suspension centrifuged at 3000 r.p.m. for 90 min. will also support growth. In the present experiments, 1 ml. of the supernatant from suspensions of liver, prepared as above and centrifuged at 2000 r.p.m. for 3 min., was added to 10 ml. of sterile 0·75 % NaCl, and the liquid sealed with vaseline. No growth of the axenic *E. invadens* occurred in this medium, even if a reducing agent, thiomalic acid, was added to the saline in which the liver was suspended, the pH of the saline-thiomalic acid being first adjusted to 6·1.

The effect of heat treatment on the growth-promoting properties of the liver suspension was also investigated by Miller, who found that heating at 50° C. for 30 min. was without effect, whereas medium heated at 56° C. for 30 min. supported less abundant growth than the untreated medium. In the present experiments, heating at 50° C. for 30 min. sometimes affected the growth-promoting properties of the saline-liver medium; thus five out of nine tubes of medium, taken from batches heated on four different occasions, supported growth of strain N, although growth was lighter than in untreated medium. Growth also occurred in saline-liver heated at 50° C. for 1 hr., and very slight growth occurred in two out of seven tubes of medium heated at 60° C. for 30 min.

Autoclaved liver extracts did not support growth, nor did combined autoclaved and Seitz-filtered extracts of liver in saline.

The growth-promoting properties of alcoholic extracts of rat liver were also tested. Alcoholic liver extracts, prepared in test-tubes according to the method described by Nelson (1947), were resuspended in 0·75 % NaCl, saline-thiomalic acid (pH 6·1), an autoclaved extract of liver in saline, and a Seitz-filtered extract of liver in saline. Starch was added to all tubes of these preparations, which, after inoculation with amoebae of strain N, were incubated for 3 weeks in an anaerobic jar containing nitrogen. No growth occurred in any of these preparations.

Tissues other than liver. Miller (1953) reported that chicken breast muscle and

rat heart muscle would support growth of axenic cultures of *E. invadens*. The ability of tissues other than liver to support the growth of strain N of *E. invadens* was therefore investigated. The media used for these tests were prepared in a similar way to the saline-liver medium; the organ to be tested was removed aseptically, and portions added to tubes containing 10 ml. of sterile 0·75% NaCl. Starch was added to each tube of culture medium. If growth occurred in any of the test cultures, the amoebae were subcultured into a new tube of medium containing the tissue under test; these second subcultures were examined for amoebic growth after 4 weeks' incubation. The results obtained are given in Table 3.

Table 3. *The ability of tissues other than liver to support the growth of an axenic strain of* Entamoeba invadens

Tissue	No. of positive cultures/no. of tests	
	1st subculture	2nd subculture
Rat kidney	2/3	0/2
Rat heart muscle	3/4	1/3
Rat lung	1/1	0/1
Rat spleen	1/3	0/1
Rat diaphragm muscle	1/1	0/1
Chick brain	1/3	—

Growth, when it occurred, was less heavy in cultures containing rat kidney, lung or spleen, or chick brain, than in medium containing rat liver, whereas rat heart or diaphragm muscle supported approximately as heavy growth as did rat liver. These limited experiments indicate, however, that continued subculture is possible only in the presence of liver. In all tissues, except lung, the amoebae resembled those produced in axenic cultures in saline-liver medium, being vacuolated, motile and containing ingested starch. The amoebae which grew in saline containing lung were very striking in appearance; exceptionally large amoebae were produced in this culture, but they were otherwise normal, and moved actively and contained large amounts of ingested starch.

Other factors. It had previously been observed that the addition of starch to the standard saline-liver medium does not influence the growth of strain N (McConnachie, 1956), although the amoebae readily ingest the starch.

Attempts were made to enhance the growth of the axenic amoebae in the stock saline-liver medium by substituting various nutrients for the 0·75% NaCl, but amoebic growth was no heavier in the enriched media than in the stock medium. The following solutions were tested—ox heart broth (Price, 1947); nutrient broth-yeast extract (0·8 g. Bacto nutrient broth, 1·0 g. Bacto yeast extract, 100 ml. H_2O); serum-saline (1 ml. inactivated horse serum + 7 ml. 0·75% NaCl); 2% peptone (trypticase, Baltimore Biological Laboratory). The pH of all these media fell within the range 6·0–7·0.

In addition, amoebic growth was not improved by the addition to the stock medium of heat-killed *Esch. coli*, autoclaved cultures of *Esch. coli* in peptone water, autoclaved, washed cells of *Esch. coli* concentrated by centrifugation from large scale cultures, and Seitz- or glass-filtered extracts of cultures of *Esch. coli* in HSre.

VI. DISCUSSION

The saline-liver medium which supports the axenic growth of *Entamoeba invadens* obviously lacks some of the growth factors supplied by bacteria in the conventional poly- and monobacterial cultures of *E. invadens*; growth in the axenic cultures is maintained at a considerably lower level than in mixed cultures, and all attempts to stimulate growth and encystation have failed. The axenic medium, however, provides a minimum of nutrients for supporting growth in serial culture indefinitely, and, as such, may serve as a useful starting-point from which the growth requirements and factors stimulating encystation of this species of *Entamoeba* may be determined. Liver appears to be unique as a source of growth factors for *E. invadens*, but little can be deduced so far concerning the nature of the active components in this tissue; they are partially labile to heat, at 50–60° C., and to prolonged storage at 8° C., and are completely removed by Seitz- or glass-filtration and autoclaving. Further work is necessary regarding the effect of centrifugation on liver extracts; centrifuged extracts of liver did not support the growth of strain N, but Miller's strain of *E. invadens* grew in the supernatant of a centrifuged suspension of liver. A brief report by Stoll (1957) also indicates that an axenic strain of *E. invadens* can be maintained in the absence of intact tissue. Stoll's medium contains an autoclaved infusion of liver or heart, together with an acidified, Seitz-filtered extract of liver, and it would appear that this medium is free of all metabolizing particles.

It is not clear at present whether the effect of pH on the growth of *E. invadens* in the axenic saline-liver medium is due to the inactivation of essential nutrients in an alkaline medium, or to the effect of an alkaline pH on the amoebae when growing in this medium. Amoebae grow in alkaline media, in mixed cultures of *E. invadens* and bacteria, but it is possible that enzymes required to use nutrients in the axenic medium have an optimum pH of 6·0, and are inactivated above pH 7·0. It is interesting that a similar pH effect has been observed with strains of *E. histolytica* (Shaffer, 1953); the optimum pH for strains growing in Shaffer-Frye medium was 6·0, with little growth above 7·0, whereas in chick-embryo tissue-bearing medium, good growth occurred above 7·0.

Several attempts have been made to isolate strains of *E. histolytica* in the axenic saline-liver medium, using both invasive and non-invasive strains of *E. histolytica*, and techniques identical to those used in the isolation of strains N and BN, but all have failed. In addition, it has not been possible to obtain axenic strains of *E. ranarum* and *E. moshkovskii* by these methods. With all three species, the amoebae failed to grow in the saline-liver medium as soon as their accompanying bacterial flora had been eliminated with antibiotics. While it is not surprising that the pathogenic *E. invadens* should differ in its growth requirements from the non-pathogenic *E. ranarum* and *E. moshkovskii*, or from a non-invasive strain of *E. histolytica*, it is of considerable interest that an invasive strain of *E. histolytica* failed to grow in the axenic saline-liver medium; it has long been known that *E. histolytica* can produce abscesses in the liver, in the absence of bacteria (Cleveland & Sanders, 1930). The nutritional differences between *E. invadens* and invasive strains of

E. histolytica are presumably greater than might be deduced from observations of their behaviour in their respective hosts.

Within the last few years, marked success has been achieved in the axenic cultivation of *E. histolytica* by other methods. Shaffer and his colleagues have developed a method by which strains can be maintained in a medium containing chick embryo tissue (Shaffer & Sienkiewicz, 1952; Shaffer, 1953; Shaffer, Sienkiewicz & Washington, 1953). A cell-free medium in which the multiplication of *E. histolytica* in micro-culture can be studied has been used by Baernstein, Rees & Bartgis (1956), and an essentially cell-free medium, based on chick embryo tissue juice, is described by Reeves, Meleney & Frye (1957*a*, *b*). *E. histolytica* has so far failed to grow in the axenic medium developed for the cultivation of *E. invadens*, but it would be of interest to discover whether *E. invadens* would grow in the media devised for the axenic cultivation of *E. histolytica*.

Even after prolonged growth in axenic culture, the amoebae of *E. invadens* have retained their ability to grow and undergo mass encystation when reassociated with suitable bacteria. Monobacterial strains prepared from strain N, by inoculating amoebae into HSre+S containing a 24 hr. culture of the required bacterium, include strains in which *E. invadens* has been associated with *Esch. coli*, organism *x* (an unidentified bacterium, isolated by McConnachie, 1956), *Bacillus subtilis*, *Aerobacter aerogenes* and *Proteus vulgaris*; of these species, *Esch. coli* and organism *x* have been most favourable for amoebic growth and mass encystation. Maintenance of axenic strains of *E. invadens* has reduced the routine maintenance of monobacterial strains to a minimum, as they can be prepared at any time, when required, from the axenic strains.

VII. SUMMARY

1. Axenic strains of *Entamoeba invadens* can be maintained indefinitely, by serial culture, in saline containing a slice of liver. Growth in this medium is poor, the amoebae are vacuolated, and few cysts are formed.

2. The optimum concentration of NaCl for amoebic growth in this medium is 0·75–1·0 %, and the optimum pH is 5·7–6·1.

3. The source of the liver tissue in the medium is unimportant, but, of several tissues tested, liver was the only one which supported amoebic growth in serial culture.

4. Suspensions of minced liver also supported amoebic growth, but the growth-promoting activity of the liver was removed completely by centrifugation, autoclaving, and Seitz- or glass-filtration. Heat treatment at 50–60° C. and prolonged storage at 8° C. partially inactivated the liver.

5. Growth in axenic saline-liver medium does not affect the ability of *E. invadens* to grow and undergo mass encystation when reassociated with suitable bacteria.

I am indebted to Dr A. Bishop for her interest and criticism, Dr R. A. Neal, of the Wellcome Laboratories of Tropical Medicine, London, for strains of *E. invadens*, *E. histolytica* and *E. moshkovskii*, and the Baltimore Biological Laboratories for a sample of trypticase.

REFERENCES

BAERNSTEIN, H. D., REES, C. W. & BARTGIS, I. L. (1956). The reproduction of *Entamoeba histolytica* with cell-free supernates of chick embryo mince. *Amer. J. Trop. Med. Hyg.* **5**, 373.

BALAMUTH, W. & SANDZA, J. G. (1944). Simple, standardized culture medium for physiological studies on *Entamoeba histolytica*, *Proc. Soc. exp. Biol., N.Y.*, **57**, 161.

CLEVELAND, L. R. & SANDERS, E. P. (1930). The production of bacteria-free amoebic abscesses in the liver of cats and observations on the amoebae in various media with and without bacteria. *Science*, **72**, 149.

DOBELL, C. & LAIDLAW, P. P. (1926). On the cultivation of *Entamoeba histolytica* and some other entozoic amoebae. *Parasitology*, **18**, 283.

DOUGHERTY, E. C. (1953). Problems of nomenclature for the growth of organisms of one species with and without associated organisms of other species. *Parasitology*, **42**, 259.

LAMY, L. (1948*a*). Obtention simple d'une culture pure temporaire d'amibes parasites. *C.R. Acad. Sci., Paris*, **226**, 1400.

LAMY, L. (1948*b*). Obtention d'une culture bactériologiquement pure d'amibes parasites pathogènes (*Entamoeba invadens*, Rodhain) ne comportant aucune addition de germes bactériens morts, ni d'aucun extrait microbien. *C.R. Acad. Sci., Paris*, **226**, 2021.

MCCONNACHIE, E. W. (1955). Studies on *Entamoeba invadens* Rodhain, 1934 *in vitro*, and its relationship to some other species of *Entamoeba*. *Parasitology*, **45**, 452.

MCCONNACHIE, E. W. (1956). Modification and elimination of the bacterial flora in cultures of *Entamoeba invadens* Rodhain, 1934. *Parasitology*, **46**, 117.

MILLER, M. J. (1951). A method for *in vitro* culture of *Entamoeba invadens* free from bacteria. *Canad. J. Comp. Med.* **15**, 268.

MILLER, M. J. (1953). Bacteria-free *Entamoeba invadens*. *Nature, Lond.*, **172**, 1192.

NELSON, E. C. (1947). Alcoholic extract medium for the diagnosis and cultivation of *Endamoeba histolytica*. *Amer. J. Trop. Med.* **27**, 545.

PRICE, W. H. (1947). Bacteriophage formation without bacterial growth. I. Formation of Staphylococcus phage in the presence of bacteria inhibited by penicillin. *J. Gen. Physiol.* **31**, 119.

REEVES, R. E., MELENEY, H. E. & FRYE, W. W. (1957*a*). Bacteria-free cultures of *Entamoeba histolytica*. *Amer. J. Trop. Med. Hyg.* **6**, 394.

REEVES, R. E., MELENEY, H. E. & FRYE, W. W. (1957*b*). Bacteria-free cultures of *Entamoeba histolytica* with chick embryo tissue juice. *Z. Tropenmed. u. Parasitol.* **8**, 213.

SHAFFER, J. G. (1953). Factors affecting the propagation of *Endamoeba histolytica in vitro* in the S–F medium and in tissue-bearing substrate. *Ann. N.Y. Acad. Sci.* **56**, 1033.

SHAFFER, J. G. & SIENKIEWICZ, H. S. (1952). Propagation of a strain of *Endamoeba histolytica* in tissue-bearing culture. *Science*, **116**, 306.

SHAFFER, J. G., SIENKIEWICZ, H. S. & WASHINGTON, J. E. (1953). The propagation of *Endamoeba histolytica* in tissue-bearing culture without accompanying bacteria or other microorganisms. *Amer. J. Hyg.* **57**, 366.

STOLL, N. R. (1957). Axenic culture of *Entamoeba invadens* in the absence of tissue. *J. Protozool.* **4**, Suppl. 8.

VAN WAGTENDONK, W. J., MILLER, C. A. & CONNER, R. L. (1952). Growth requirements of *Paramecium aurelia*, var. 4, stock 51·7(s) in a medium free of other living organisms. *Fed. Proc.* **11**, 302.

ON THE MORPHOLOGY OF THE MALE, FEMALE AND FOURTH-STAGE LARVA (FEMALE) OF *HAMMERSCHMIDTIELLA DIESINGI* (HAMMERSCHMIDT), A NEMATODE PARASITIC IN COCKROACHES

By D. L. LEE

The Molteno Institute, University of Cambridge

(With 8 Figures in the Text)

The first oxyurid nematode reported from a cockroach was *Oxyuris diesingi*, described by Hammerschmidt (1838), from the intestine of *Blatta orientalis* L. In 1847 he described another nematode, *O. blattae orientalis* which appears to be *O. diesingi* re-described and re-named. Further descriptions were given by Bütschli (1871) and by Galeb (1878). Chitwood (1932) revised the descriptions of the nematode parasites of Blattidae and described several new species. He also created a new genus, *Hammerschmidtiella*, to contain *O. diesingi*. Serrano Sánchez (1947) described *Hammerschmidtiella neyrae* from the intestine of *Periplaneta orientalis* [? = *Blatta orientalis*]. The male and female are slightly larger than those of *H. diesingi*, as described by Chitwood (1932), whilst the male possesses a smaller pseudobulb and a shorter tail. It is possible that *H. neyrae* is a synonym or a variety of *H. diesingi* as the differences between the two are so slight.

Hammerschmidtiella diesingi (Hammerschmidt, 1838) (Chitwood, 1932), together with two other species of Thelastomatidae (Travassos, 1929), has been found in the hind-gut of *Blatta orientalis* and of *Periplaneta americana* L. kept in stock cultures in the Molteno Institute.

By feeding infective stage eggs of *Hammerschmidtiella diesingi* to uninfected *Blatta orientalis* it has been found that the nematode can be identified by the female fourth-stage larva. As the mature male and the female fourth-stage larva have an unusual structure in the intestinal wall, first noticed by Bütschli (1871) in the male, it has been decided to give descriptions of the male, female and fourth-stage larva (female), and also to describe this unusual structure in the intestine.

MORPHOLOGY

Male (Figs. 1, 2, 8). Length: 800 μ to 1·1 mm., average 980 μ. Width: 50–80 μ, average 68 μ. Oesophagus: length 125–157 μ, average 140 μ. Anterior oesophagus: 60–80 μ, average 73 μ; width 15–20 μ, average 17 μ. Bulb: width 22–28 μ, average 25 μ. Nerve ring: 70–103 μ from anterior end of body, average 90 μ. Excretory pore: 193–250 μ from anterior end of body, average 220 μ. Anus: 92–120 μ from tip of tail, average 100 μ. Spicule: 20–30 μ, average 27 μ.

Narrow lateral alae present. Corpus of oesophagus slightly swollen, long isthmus and distinct valvular bulb present. Intestine separated into two parts by a con-

striction and ring of clear cells, one cell in width (Fig. 8). Testis slightly posterior to excretory pore, opens directly into seminal vesicle. Vas deferens surrounded by large glandular cells. Tail distinctly marked off from body, filiform. Caudal papillae consisting of one pair of large preanal papillae, one pair of large adanal papillae, one pair of small adanal papillae and one large ventral papilla at base of tail (Fig. 2). One spicule.

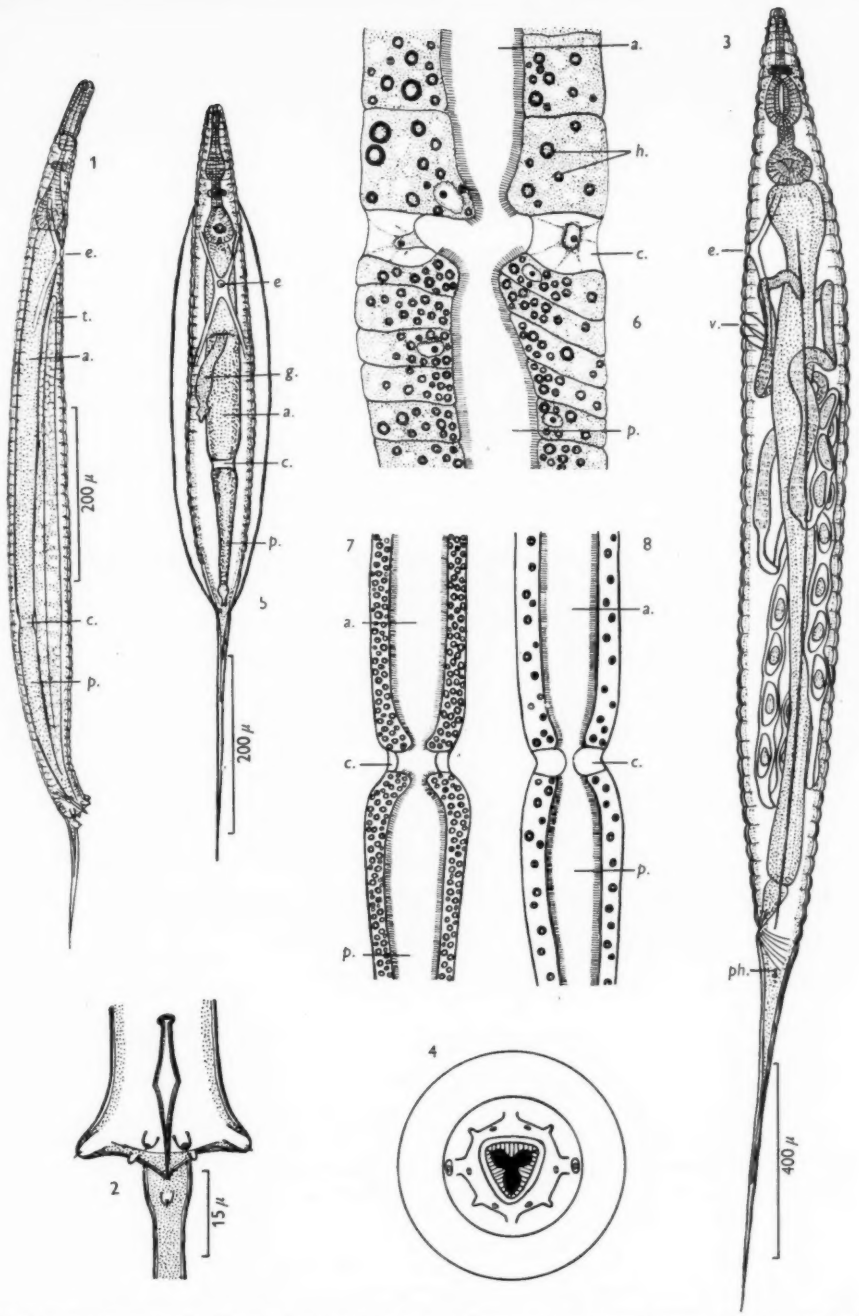
Female (Figs. 3, 4). Length: 2·0–3·6 mm., average 2·5 mm. Width: 200–282 μ, average 230 μ. Oesophagus: length 290–375 μ, average 330 μ. Anterior oesophagus: length 180–220 μ, average 203 μ; width 70–82 μ, average 72 μ. Isthmus: length 37–55 μ, average 46 μ; width 17–30 μ, average 25 μ. Bulb: width 75–95 μ, average 88 μ. Nerve ring: 100–135 μ from anterior end of body, average 120 μ. Excretory pore: 440–636 μ from anterior end of body, average 500 μ. Anus: 627 μ–1·2 mm. from tip of tail, average 924 μ. Vulva: 684–900 μ from anterior end of body, average 750 μ. Eggs: length 85–87 μ, average 86 μ; width 33–35 μ, average 35 μ.

Cuticle closely annulated throughout length of body. First six annulations well marked. Lateral alae prominent, run from base of oesophagus to base of tail, do not terminate in spines. Mouth surrounded by eight submedian papillae and six inner labial papillae, amphids present (Fig. 4). Corpus terminated by large ovoid pseudobulb. Isthmus long and valvular bulb distinct. Excretory pore posterior to base of oesophagus. Anterior part of intestine enlarged to form a cardia, no clear cells present. Vulva in anterior part of body. Two ovaries, both originating in anterior third of body. Tail long and filiform. Eggs ellipsoidal, deposited in two- or four-cell stage.

Fourth-stage larva (*female*) (Figs. 5–7). Length: 700 μ to 1·1 mm. Width: 75 μ. Prominent lateral alae present. Anterior part of oesophagus slightly swollen; valvular bulb and isthmus distinct. Intestine separated into two parts by a constriction and ring of clear cells, one cell in width (Figs. 6, 7). Excretory pore posterior to base of oesophagus. Genital primordium slightly anterior to middle of body. Tail long and filiform.

The intestine of the male and of the fourth-stage larva (female), as described above, is separated into two parts by a ring of clear cells, one cell in width, which completely encircles the intestine thus forming a 'waist'. It has been found that the intestinal cells contain inclusion bodies which stain heavily with haematoxylin, few in the mature male but numerous in the fourth-stage larva (Figs. 6–8). These inclusion bodies, however, are not present in the clear cells. In the fourth-stage larva the cells adjacent to the clear cells project into the lumen, whereas in the male the clear cells themselves project into the lumen (Figs. 6–8). The rod border (bacillary layer) is continuous over the inner surface of the normal intestinal cells, but is not present over the inner surface of the clear cells.

This area of the intestine has been observed, in the living fourth-stage larva, to act as a sphincter. Normally this sphincter is open allowing free movement, from anterior to posterior intestine, of the intestinal contents which oscillate in the lumen as a result of body movements. Definite movement down the intestine is brought about by contraction in diameter of the intestine, together with the action



Figs. 1–8 *Hammerschmidtiella diesingi*. Fig. 1. Mature male, lateral view. Fig. 2. Tail of male, ventral view. Fig. 3. Mature female, lateral view. Fig. 4. Head of female, *en face* view. Fig. 5. Fourth-stage larva (female), ventral view. Fig. 6. Longitudinal section of intestine of fourth-stage larva (female) in region of clear cells, lateral view. Fig. 7. Diagram of intestine of fourth-stage larva in region of clear cells. Fig. 8. Diagram of intestine of male in region of clear cells. *a.*, anterior intestine; *c.*, clear cells; *e.*, excretory pore; *g*, genital primordium; *h.*, haematoxylin-stained inclusion bodies; *p.*, posterior intestine; *ph*, phasmid; *t.*, testis; *v.*, vulva.

of the sphincter. The intestinal contents are passed along the anterior into the posterior intestine; the ring of clear cells constricts, separating the two portions of the intestine by closing the lumen, and the contents are then passed along the length of the posterior intestine. However, when the sphincter opens some forward movement occurs.

In the male, when contraction of the intestine takes place, movement of the intestinal contents along the lumen is brought to a halt at the constriction by closing of the sphincter. This closing is brought about by contraction in diameter of the posterior intestine adjacent to the clear cells; as a result, the contents of the posterior intestine are driven towards the anus. This mechanism is brought into operation in the male, also in the larva, when the contents of the posterior intestine are passed out of the anus. When there is no definite movement along the intestine the sphincter is open, as in the fourth-stage larva. However, the intestinal contents which move up and down the lumen as a result of body movements are filtered at the constriction by the clear cells which project into, and so narrow, the lumen, thus allowing only the smaller particles through into the posterior intestine.

The living larvae and adults were studied in 12% sea water (Hobson, 1948). All measurements were made upon heat-killed nematodes.

SUMMARY

The male, female and fourth-stage larva (female) of *Hammerschmidtiella diesingi* are described. Characters are given by which this species can be identified from the female fourth-stage larva.

A constriction and ring of clear cells separate the intestine of the male and of the female fourth-stage larva into two parts, and descriptions of the structure and mode of action of this 'sphincter' are given.

This work was carried out during the tenure of a Research Studentship awarded by the Department of Scientific and Industrial Research.

REFERENCES

Bütschli, O. (1871). Untersuchungen über die beiden Nemtoden der *Periplaneta* (*Blatta*) *orientalis* L. *Z. wiss. Zool.* **21**, 252–93.

Chitwood, B. G. (1932). A synopsis of the nematodes parasitic in insects of the family Blattidae. *Z. Parasitenk.* **5**, 14–50.

Galeb, O. (1878). Recherches sur les entozoaires des insectes. Organisation et développement des oxyuridés. *Arch. Zool. exp. gén.* **7**, 283–390.

Hammerschmidt, K. E. (1838). Helminthologische Beiträge. *Isis* (*Oken*), *Leipzig*, **5**, 351–8.

Hammerschmidt, K. E. (1847). Helminthologische Beiträge. Beschreibung einiger *Oxyuris*-Arten. *Naturw. Abh., Wien*, **7**, 279–92.

Hobson, A. D. (1948). The physiology and cultivation in artificial media of nematodes parasitic in the alimentary tract of animals. *Parasitology*, **38**, 183–227.

Sánchez, A. Serrano (1947). Nematodes parasitos de los artropods en España. *Rev. ibér. Parasit.* **7**, 279–332.

Travassos, L. (1929). Contribuição preliminar á systematica dos nematodeos dos arthropods. *Mem. Inst. Osw. Cruz* (Suppl. 5), pp. 19–25.

DIGESTION IN *LEIDYNEMA APPENDICULATA* (LEIDY, 1850), A NEMATODE PARASITIC IN COCKROACHES

By D. L. LEE

The Molteno Institute, University of Cambridge

(With 4 Figures in the Text)

The most complete studies of the digestive enzymes in nematodes are by Rogers (1940, 1941*a*, *b*) and by Carpenter (1952). Rogers detected an amylase, a proteinase, a lipase and an esterase in a glycerin suspension of the intestine of *Strongylus edentatus* and of *Ascaris lumbricoides* var. *suis*. These results were confirmed, for *A. lumbricoides*, by Carpenter who also found that four peptidases were present.

As Rogers and Carpenter worked with nematodes found in the intestine of vertebrates, it was decided to determine the nature of the enzymes of a nematode found in the intestine of an insect and to compare the results with those obtained by Rogers and Carpenter for *A. lumbricoides* and *S. edentatus*.

The nematode selected was *Leidynema appendiculata* (Leidy, 1850) (Chitwood, 1932), which is parasitic in the hind-gut of the cockroaches *Blatta orientalis* L. and *Periplaneta americana* L. kept in stock cultures in the Molteno Institute. This nematode feeds upon the contents of the hind-gut of the host.

PREPARATION OF THE ENZYME MATERIAL

The heads were cut off mature *L. appendiculata* females (average length 3 mm.), just anterior to the oesophageal bulb, and the tails were cut off slightly anterior to the anus. These operations were carried out in distilled water, under a binocular microscope, using needle-point forceps and a surgeon's needle with a cutting edge. The intestines, together with the oesophageal bulbs, were drawn out of the body of the nematodes by means of the fine forceps, washed in distilled water to remove traces of body fluid and placed on the grinding surface of the pestle of a small glass homogenizer. The guts of 30 nematodes (20 for amylase experiments) were ground in the small homogenizer with 0·01 ml. of 50% glycerin for each experiment.

The buffer solutions (distilled water in carbohydrase experiments) used in the experiments were added to the glycerin extract in the homogenizer and used to wash the pestle free of extract. This glycerin extract was boiled to inactivate the enzyme before incubation in all control experiments.

The buffer solutions used throughout the following experiments were the phosphate and phthalate buffers of Clark & Lubs as quoted by Cole (1955). Toluol was added to every tube, in the following experiments, to prevent bacterial growth.

CARBOHYDRASES

Amylase

Gut extract was incubated with 2% starch solution and buffer solution pH 6·0 at 30° C. for 24 hr. After incubation, positive results were obtained when the reaction mixture was boiled with Benedict's solution whilst control tubes gave negative results. This experiment indicated the presence of an amylase in the gut extract of *Leidynema*.

The effect of hydrogen-ion concentration and of NaCl

Method. The pH at which optimum activity takes place was determined by estimating the amount of reducing sugar produced by the digestion of a starch solution at various hydrogen-ion concentrations.

Distilled water, 0·20 ml., was added to 0·01 ml. of glycerin extract as prepared above. Small test tubes (10 by 50 mm.) containing 0·03 ml. of this dilute extract, 0·05 ml. of a 2% starch solution and 0·05 ml. of buffer, with 0·01 ml. of distilled water and 0·01 ml. of 0·05 M sodium chloride (NaCl) in alternate tubes, were shaken in a water-bath at 30° C. for 1 hr. In each experiment there were six experimental tubes and one control tube. The reducing sugar produced was estimated by a modification of Hagedorn & Jensen's method of determining blood sugar as described by Cole (1955) and using the table given by Hagedorn & Jensen (1923).

TABLE 1. *The amounts of reducing sugar produced by gut extract of* Leidynema appendiculata *from a* 2% *starch solution, with and without* NaCl *added, and the percentage increase in activity, due to the* NaCl, *at each* pH *value*

	Reducing sugar (mg. %)		
pH	NaCl not added	NaCl added	Percentage increase in activity
4·0	41	52	26
4·5	77	99	29
5·0	68	95	39
5·5	59	83	40
6·0	17	39	130
7·0	5	—	—

Results. Optimum activity was obtained at pH 4·5. It was found that sodium chloride increased the activity of the amylase, but this increase in activity was greater on the alkaline side of pH 4·5 than on the acid side. The amounts of reducing sugar (in mg. %) produced from the starch are shown in Table 1 and relative velocities are shown in Fig. 1. In the estimation of reaction velocities the hydrolysis of starch is assumed to be a linear function of time, and therefore the velocity is directly proportional to the amount of reducing sugar produced in a given time (Wigglesworth, 1927*b*).

Wigglesworth (1927*b*) found that cockroach maltase and lactase were completely inhibited by 10% glycerin and the invertase strongly inhibited by 20% glycerin. It was therefore decided to use aqueous extracts of the gut of *L. appendiculata* in

the experiments to detect maltase, lactase and invertase. The guts of twenty adult females were ground with 0·01 ml. of distilled water in a small homogenizer and then diluted with 0·20 ml. of distilled water.

Maltase

Using osazone tests, maltase was shown to be present in gut extract by incubating it with 4 % maltose solution at 30° C. for 24 hr. and then examining the crystals microscopically when glucosazone and maltosazone were found. Only maltosazone was found in the control tubes.

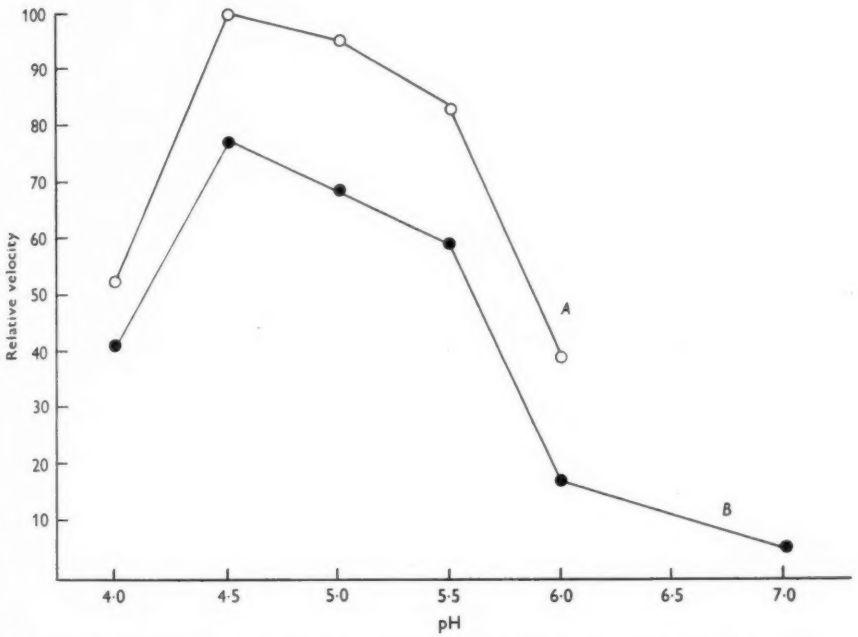


Fig. 1. The relative velocity of the amylase from the gut of *L. appendiculata* at different hydrogen-ion concentrations. *A*, NaCl added; *B*, NaCl not added.

The effect of hydrogen-ion concentration and of glycerin

Method. Small test tubes containing 0·03 ml. of the dilute extract, 0·05 ml. of 0·5 % maltose and 0·05 ml. of buffer, with 0·02 ml. of distilled water and 0·02 ml. of glycerin in alternate tubes, were shaken in a water-bath at 30° C. for 24 hr. In each experiment there were six experimental tubes and one control tube containing boiled extract. The glucose produced was estimated by a modification of Hagedorn & Jensen's method of determining blood sugar as described by Cole (1955).

Results. Maximum activity was obtained at pH 5·0–6·0. It was found that the presence of glycerin in a concentration of 15 % completely inhibited activity of the

maltase. The amounts of glucose (in mg. %) produced from the maltose are shown in Table 2, and relative activity of the maltase at various hydrogen-ion concentrations is shown in Fig. 2.

TABLE 2. *The amounts of glucose produced by gut extract of* Leidynema appendiculata *from* 0·5% *maltose*

pH	Glucose (mg. %)
3·0	7
4·0	17
4·5	25
5·0	34
6·0	33
7·0	16
8·0	0

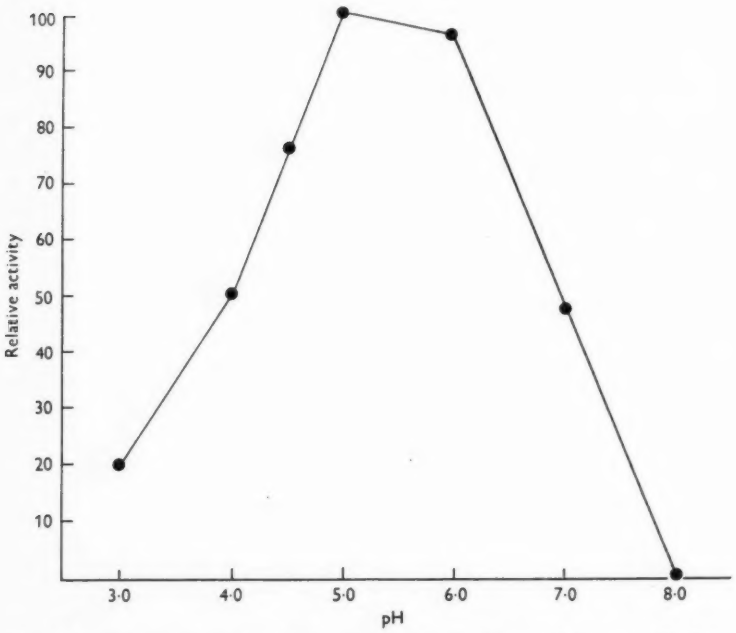


Fig. 2. The relative activity of the maltase from the gut of *L. appendiculata* at different hydrogen-ion concentrations.

Lactase

Negative results were obtained when gut extract was incubated with a 4% lactose solution at 30° C. for 24 hr. and then tested for glucosazone. However, Evans (1956) found that glucosazone crystals cannot be prepared from reaction mixtures containing two or more sugars when the concentration of glucose present is relatively low, therefore, a negative osazone test does not necessarily indicate the non-hydrolysis of the substrate.

The experiments to determine the effect of hydrogen-ion concentration and of glycerin on maltase activity were repeated substituting a 0·5 % lactose solution for the 0·5 % maltose. All results were negative, indicating that no lactase is present.

Invertase

Gut extract was incubated with 10 % sucrose solution at 30° C. and then tested for the presence of reducing sugars, using Benedict's solution. After 48 hr. incubation there was no reduction of the Benedict's solution.

The experiments to determine the amount of glucose produced from maltose by the maltase were repeated with 0·5 % sucrose solution in place of the maltose. No reduction of the sucrose to glucose was detected, showing that the gut extract lacks an invertase.

NATURE OF THE SUGARS FORMED BY DIGESTION OF STARCH

After 24 hr. incubation at pH 4·5, the products of digestion of a 1 % starch solution by gut extract were examined as osazones. Only glucosazone crystals were found, indicating that *L. appendiculata* breaks down starch to glucose.

PROTEASES

Gut extract incubated at 30° C. with calcified milk produced a clot in 20 min. indicating the presence of a proteolytic enzyme. The clot was not digested after 7 days' incubation. Control tubes containing calcified milk and boiled extract did not form clots.

The effect of hydrogen-ion concentration.

Method. The pH for optimum activity of the enzyme has been determined by using Blacklock, Gordon & Fine's (1930) modification of the Henriques-Sørensen 'formol titration' method for the estimation of amino-acid.

Small test tubes containing an extract of thirty guts in 0·01 ml. of 50 % glycerin, 0·09 ml. of buffer and 0·1 ml. of 4 % gelatin or 4 % edestin were shaken in a water-bath at 30° C. Control tubes contained boiled extract. After 48 hr., 0·1 ml. was removed from the tube and added to 5 ml. of distilled water in a comparator tube, 5 drops of B.D.H. phenolphthalein were added as indicator and N/100 NaOH was run in from a 1 ml. burette, to which was attached a capillary termination giving a drop of 0·01 ml., until the first trace of pink appeared; 0·5 ml. of 'neutral formol' was added and the mixture titrated with N/100 NaOH to the first trace of pink, using the microburette. The 'formol acidity' figure was obtained by subtracting the amount of NaOH required by the contents of the control tube from the amount required by the contents of the experimental tube.

Results. Maximum activity was obtained at pH 6·0. The amounts of 'formol acid' found are shown in Table 3 and relative activity is shown in Fig. 3. Less 'formol acid' was produced when edestin was the substrate than when gelatin was the substrate.

As the optimum pH of the protease is in the pH range of the cathepsin enzymes, it was decided to see if a cathepsin activator would increase activity.

The experiments to determine the amounts of 'formol acid' produced by the protease from 4% gelatin were repeated, but with 0·01 ml. of 0·036 M potassium cyanide (KCN) and 0·01 ml. of distilled water in alternate tubes. Results showed that KCN does not activate the protease present in the gut of *L. appendiculata*.

TABLE 3. *The amounts of* N/100 *'formol acid' produced by gut extract of* Leidynema appendiculata *from* 4% *gelatin and* 4% *edestin*

	N/100 'formol acid' (ml.)	
pH	4% gelatin	4% edestin
4·0	0·035	0·020
5·0	0·060	0·040
5·5	0·080	0·050
6·0	0·110	0·070
6·5	0·085	0·040
7·0	0·040	—

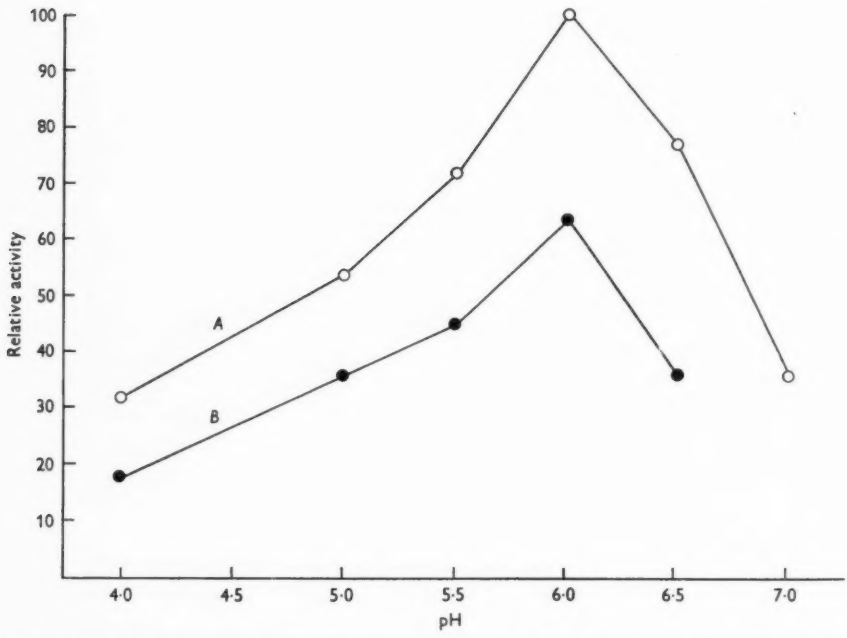


Fig. 3. The relative activity of the protease from the gut of *L. appendiculata* at different hydrogen-ion concentrations. *A*, gelatin as substrate; *B*, edestin as substrate.

PEPTIDASES

The quantitative method of determining protease activity was used to detect any peptidase activity, substituting 0·1 ml. of peptide solution for the 0·1 ml. of gelatin. The amount of 'formol acid' produced at pH 6·0 was then estimated.

The substrates used were the dipeptides glycyl-glycine (0·2 M) and DL-leucyl-

glycine (saturated solution, 0·18 M); and the tripeptide DL-leucyl-glycyl-glycine (0·2 M). It was found that peptidases capable of splitting these three peptides were present (Table 4). A prolidase capable of splitting 0·2 M glycyl-L-proline was also shown to be present (Table 4).

TABLE 4. *The amounts of* N/100 *'formol acid' produced by gut extract of* Leidynema appendiculata *from two dipeptides, a tripeptide and a proline at* pH 6·0

Substrate	N/100 'formol acid' (ml.)
Glycyl-glycine	0·23
DL-Leucyl-glycine	0·48
DL-Leucyl-glycyl-glycine	0·12
Glycyl-L-proline	0·22

LIPASE

Determination of the pH optimum

Method. Emulsions of olive oil and of glycerol tributyrate were prepared, using gum arabic (Fodor, 1948). Instead of the 10 ml. of phosphate buffer used by Fodor, 5 ml. of distilled water were used.

Small test tubes containing 0·01 ml. of glycerin extract, 0·09 ml. of buffer solution and 0·1 ml. of substrate were shaken in a water-bath at 30° C. for 48 hr. Control tubes contained boiled extract. After incubation, 0·1 ml. of the reaction mixture was added to 5 ml. of distilled water in a comparator tube and titrated against N/100 NaOH to the phenolphthalein end point. The burette was a 1 ml. microburette to which was attached a capillary termination giving a drop of 0·01 ml. The amount of fatty acid produced in the experimental tube was determined by subtracting the amount of N/100 NaOH required by the control tube from that required by the experimental tube.

TABLE 5. *The amounts of* N/100 *fatty acid produced by gut extract of* Leidynema appendiculata *from glycerol tributyrate emulsion*

pH	N/100 fatty acid (ml.)
5·0	0
5·5	0·010
6·0	0·033
7·0	0·042
8·3	0·018

Results. With olive oil emulsion as the substrate no positive results were obtained. However, with tributyrate emulsion there was definite hydrolysis with maximum activity at pH 7·0. The amounts of N/100 fatty acid produced are shown in Table 5 and relative activity in Fig. 4.

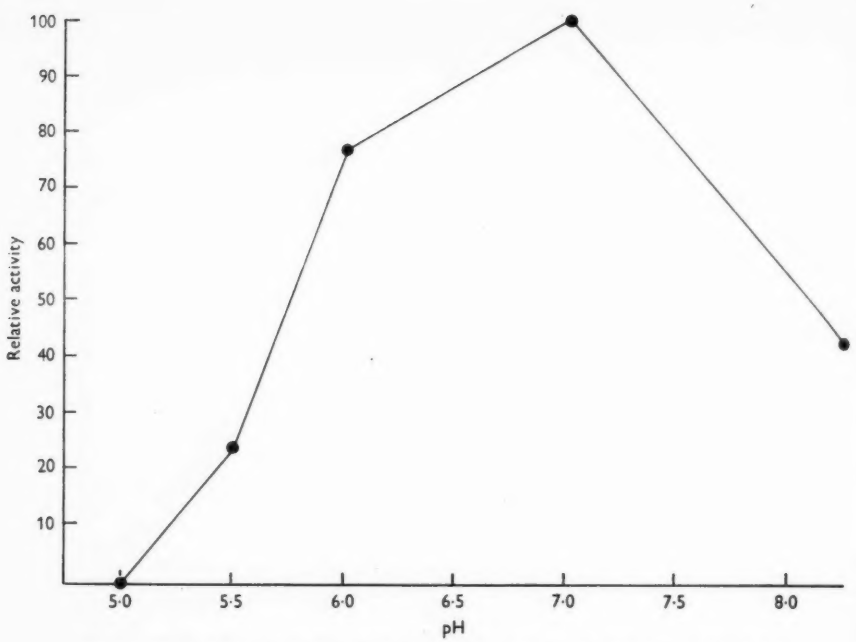


Fig. 4. The relative activity of the lipase from the gut of *L. appendiculata* at different hydrogen-ion concentrations.

ESTERASE

Using the method described for lipase detection, with a gum arabic emulsion of ethyl butyrate in place of the tributyrate emulsion, no positive results were obtained.

DISCUSSION

Following Wigglesworth's (1927*a*) technique the hydrogen-ion concentration of the hind-gut of *P. americana* was found to be approximately pH 7·0, and it was thought that the digestive enzymes of *L. appendiculata* would function at a similar hydrogen-ion concentration. However, it has been shown that this is not so.

The amylase in *L. appendiculata* has maximum activity at pH 4·5 and functions in a more acid medium than is found in the hind-gut of the cockroach; however, it may be an intracellular enzyme which would possibly explain the low optimum pH. Thus, this amylase differs from that found in the cockroach, optimum pH 5·9 (Wigglesworth, 1927*b*), and from the amylases found in the gut of *S. edentatus*, optimum pH 8·0 (Rogers, 1940), and *A. lumbricoides*, optimum pH 9·3–9·4 (Rogers, 1940; Carpenter, 1952). It has been found that the amylase of *L. appendiculata* resembles that of other animals in that it is activated by NaCl, there being 29% activation with NaCl at pH 4·5. Carpenter (1952) found there was 30% activation of the amylase of *A. lumbricoides* with NaCl at the optimum pH (9·4). The percentage activation of the amylase of *Leidynema* with NaCl is greater

on the alkaline side than on the acid side of the optimum pH, thus, whilst there is 26% increase in activity at pH 4·0, there is 39% increase at pH 5·0.

Carpenter (1952) demonstrated the existence of a maltase, with maximum activity at pH 8·0, in the gut extract of *A. lumbricoides* and Wigglesworth (1927*b*) showed that there is a maltase, optimum pH 5·0–6·2, in the hepatic caeca of the cockroach. The maltase of *Leidynema* closely resembles that of the cockroach, having a similar optimum pH (5·0–6·0); this enzyme is not host enzyme which has been ingested with the food of the nematode, however, for no maltase was found to be present in the hind-gut contents. It has been found that, as with cockroach maltase (Wigglesworth, 1927*b*), there is complete inhibition of the maltase of *Leidynema* with 15% glycerin. It is of interest to note that whilst maximum activity of the maltase of *Ascaris* takes place at pH 8·0, i.e. at a slightly higher hydrogen-ion concentration than the optimum pH of the amylase (pH 9·4), maximum activity of the maltase of *Leidynema* occurs at pH 5·0–6·0, i.e. at a slightly lower hydrogen-ion concentration than the optimum pH of the amylase (pH 4·5).

Although Wigglesworth (1927*b*) has shown that there is a lactase and an invertase in the cockroach, neither of these enzymes has been detected in *Leidynema*. This is further evidence that the maltase found in the nematode is not ingested host enzyme. Rogers (1940) found there was only very slight invertase activity, if any, in the gut of *A. lumbricoides* and of *S. edentatus*.

The protease of *Leidynema*, with optimum activity at pH 6·0, is similar to that of *Ascaris*, with optimum activity at pH 6·0–6·2 (Carpenter, 1952; Rogers, 1941*b*), and of *Strongylus*, with optimum activity at pH 6·2 (Rogers, 1941*b*), but differs from that of the cockroach which has optimum activity at pH 7·5–8·0, depending upon the substrate (Wigglesworth, 1928). Although the optimum pH of the protease is similar to that of some cathepsins, unlike them it is not activated by KCN, and in this it resembles the protease of *Ascaris* (Carpenter, 1952) and of the cockroach (Wigglesworth, 1928), neither of which is activated by KCN. The protease of *Leidynema* has been shown to be more efficient in splitting gelatin than edestin, but optimum activity for both substrates takes place at the same hydrogen-ion concentration, whereas the protease of the cockroach has maximum activity at pH 7·5 with gelatin as the substrate and at pH 8·0 with edestin as the substrate (Wigglesworth, 1928).

Carpenter (1952) found that four peptidases were present in the gut extract of *Ascaris*, namely, leucine amino peptidase, glycyl-glycine peptidase, alanylglycine peptidase and a less specific amino peptidase. Peptidases capable of splitting glycyl-glycine, DL-leucyl-glycine and DL-leucyl-glycyl-glycine have been shown to be present in the gut extract of *Leidynema*. A prolidase capable of splitting glycyl-L-proline, believed to be an important breakdown product of gelatin and other proteins, has also been shown to be present.

Wigglesworth (1928) has demonstrated the presence of an enzyme from the hepatic caeca of cockroaches capable of hydrolysing ethyl butyrate, with maximum activity at pH 8·0. Rogers (1941*a*), using olive oil and ethyl butyrate as substrates, found that *A. lumbricoides* and *S. edentatus* both possessed a lipase and an esterase

and that maximum activity took place at pH 8·0. These results were confirmed, for *Ascaris*, by Carpenter (1952) who used methyl butyrate and glycerol tributyrate as the substrates. It has been found that the lipase of *Leidynema* will split glycerol tributyrate, but hydrolysis of olive oil and ethyl butyrate was not detected. Optimum activity of the lipase occurs at a higher hydrogen-ion concentration (pH 7·0) than those of the insect host, *A. lumbricoides* and *S. edentatus*. The negative results obtained with ethyl butyrate do not necessarily indicate the absence of an esterase, as Fodor (1948) found that emulsification of ethyl butyrate, ethyl propionate and methyl butyrate with gum arabic inhibited cleavage by 60–100%, depending upon the origin of the enzyme and the nature of the substrate, and it may be that the esterase of *Leidynema* is completely inhibited by the gum arabic.

We have seen that the enzymes shown to be present in gut extract of *L. appendiculata* are all capable of 60–80% activity at pH 5·5–6·0, except maltase which has 100% activity over this range, and it is possible that digestion in the gut of the nematode takes place over this pH range. It has also been shown that the enzymes found to be present in the nematode are quite distinct from those of the host and, except for the protease, have different pH optima from those of *A. lumbricoides* and *S. edentatus*.

SUMMARY

1. A study of the digestive enzymes present in the nematode *Leidynema appendiculata*, parasitic in the cockroach, gave the following results.

2. An amylase with optimum activity at pH 4·5, is present. Sodium chloride increases activity by 29% at this pH.

3. A maltase, with maximum activity at pH 5·0–6·0, is present. It is completely inhibited by 15% glycerin.

4. Lactase and invertase were not detected.

5. A protease, capable of clotting calcified milk, and of splitting gelatin and edestin, with maximum activity at pH 6·0, is present. KCN does not increase activity.

6. Peptidases splitting glycyl-glycine, DL-leucyl-glycine and DL-leucyl-glycyl-glycine, and a prolidase splitting glycyl-L-proline are present.

7. A lipase hydrolysing glycerol tributyrate, with maximum activity at pH 7·0 is present. Hydrolysis of olive oil and ethyl butyrate was not detected.

8. It is concluded that these enzymes are quite distinct from those of the host and that digestion probably takes place over the range of pH 5·5–6·0.

This work was carried out during the tenure of a Research Studentship awarded by the Department of Scientific & Industrial Research.

REFERENCES

Blacklock, D. B., Gordon, R. M. & Fine, J. (1930). Metazoan immunity: A report on recent investigations. *Ann. Trop. Med. Parasit.* **24**, 5–54.

Carpenter, M. F. P. (1952). The digestive enzymes of *Ascaris lumbricoides* var. *suis*; their properties and distribution in the alimentary canal. Dissertation, Univ. Michigan. Univ. Microfilms, Publ. no. 3729. Ann Arbor, Mich. 183 pp.

CHITWOOD, B. G. (1932). A synopsis of the nematodes parasitic in insects of the family Blattidae. *Z. Parasitenk.* **5**, 14–50.

COLE, S. W. (1955). *Practical Physiological Chemistry*, 10th ed. Revised by Baldwin, E. & Bell, D. J. Cambridge: Heffer.

EVANS, W. A. L. (1956). Studies on the digestive enzymes of the blowfly *Calliphora erythrocephala*. I. The carbohydrases. *Exp. Parasitol.* **5**, 191–206.

FODOR, P. J. (1948). The specific inhibition by sodium taurocholate of part of the lipolytic enzyme systems contained in extracts from various organs of the grasshopper (*Schistocerca gregaria* Forsk. and *Dociostaurus moroccanus* Thnb.). *Enzymologia*, **12**, 333–42.

HAGEDORN, H. C. & JENSEN, B. N. (1923). Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid. *Biochem. Z.* **135**, 46–58.

ROGERS, W. P. (1940). Digestion in parasitic nematodes. I. The digestion of carbohydrates. *J. Helminth.* **18**, 143–54.

ROGERS, W. P. (1941*a*). Digestion in parasitic nematodes. II. The digestion of fats. *J. Helminth.* **19**, 35–46.

ROGERS, W. P. (1941*b*). Digestion in parasitic nematodes. III. The digestion of proteins. *J. Helminth.* **19**, 47–58.

WIGGLESWORTH, V. B. (1927*a*). Digestion in the cockroach. I. The hydrogen-ion concentration. *Biochem. J.* **21**, 791–6.

WIGGLESWORTH, V. B. (1927*b*). Digestion in the cockroach. II. The digestion of carbohydrates. *Biochem. J.* **21**, 797–811.

WIGGLESWORTH, V. B. (1928). Digestion in the cockroach. III. The digestion of proteins and fats. *Biochem. J.* **22**, 150–61.

THE PHYSIOLOGY OF DIGESTION IN THE LARVAE OF THE HORSE BOT-FLY, *GASTEROPHILUS INTESTINALIS* (DE GEER)

By R. J. TATCHELL

The Molteno Institute of Biology and Parasitology, University of Cambridge

(With 8 Figures in the Text)

A great many studies have been made of the nutrition and the digestive capabilities of insects. The cockroach was the subject of a comprehensive survey by Wigglesworth (1927); and, among Diptera, of the more generalized feeders, *Calliphora* (Wigglesworth, 1929; Fraenkel, 1940; Evans, 1956) and *Lucilia* (Hobson, 1931); of the blood feeders, *Glossina* (Wigglesworth, 1929) and *Chrysops* (Wigglesworth, 1931); and of the endoparasitic forms, *Gasterophilus* (Roy, 1937) and *Hypoderma* (Simmons, 1939) have been studied in more or less detail. The experiments of Wigglesworth suggested that the digestive enzymes of any insect could be correlated with its diet. Thus, adult *Calliphora* feeding on a carbohydrate-rich diet possessed a full range of potent carbohydrases and showed reduced proteinase activity, whereas the blood-feeding flies had very active proteinases and carbohydrases were weak or absent. This view was supported by Day & Waterhouse (1953).

Evans (1956) found that larval and adult *Calliphora* had essentially similar enzyme complements despite their dietary differences. Thus a full range of carbohydrases was present in the larvae which have very little carbohydrate in the diet. He also stated that preliminary experiments on *Lucilia sericata* indicated that sucrose and trehalose could be digested by both salivary gland and mid-gut extracts in contrast to the results of Hobson (1931). Simmons (1939) showed that larvae of *Hypoderma lineatum* had a sucrase and a maltase although he could not detect an amylase. Since Roy (1937) showed that larvae of *Gasterophilus intestinalis* possessed an amylase, a proteinase and a lipase it was decided to make a more detailed study of the digestive enzymes present in this species.

DESCRIPTION OF HABITAT AND DIET OF *GASTEROPHILUS INTESTINALIS*

The larvae of *G. intestinalis* are completely endoparasitic from the time the first-instar larva emerges from the egg and attaches itself to the horse's tongue before burrowing beneath the mucosa, where the first moult occurs. The second-instar larva emerges from the tongue after about a fortnight and attaches itself to the lining of the pharynx where it hangs for a few days before passing to the stomach. The remaining 7–8 months of larval development are spent attached to the non-glandular epithelium of the horse's stomach. In the third instar the larvae are generally clumped near the boundary of the non-glandular and the glandular

epithelium. The diet of the third-instar larva has been difficult to determine. The mid-gut is generally slightly distended with a clear or opalescent pale-yellow, cell-free liquid. Dinelescu (1932) believed that the larvae ingested blood but, as he could find only 4 larvae, out of 450 dissected, with unmistakably fresh blood in their intestines and a further 13 with signs of partly digested blood, he decided that the larvae fed very infrequently and that this was responsible, in part, for the long and slow development of the larva. Roy (1937) dissected 100 larvae from a freshly killed horse and found no sign of ingested blood. In the present investigation it was noticed that second-instar larvae frequently contained freshly ingested blood in their mid-gut. It was also noticed that the third-instar larvae were, in the majority of cases, firmly attached with the anterior extremity and the oral hooks embedded in a ring-shaped swelling of the stomach wall and that when removed a distinct circular pit remained. When sectioned and stained the swollen ring and the pit were seen to be the centre of an intense inflammatory response with marked leucocytic infiltration of the surrounding fibrous tissue. In the case of second-instar larvae this response is either not provoked or the larvae do not remain in one spot long enough for its full development. Around the fringes of a clump of well-established third-instar larvae it is common to find a few larvae only loosely attached and without the associated tissue response of the stomach wall. Apparently these larvae would have only recently become attached in that position, and, on dissection, it was found that, like the second-instar larvae, 50 % or more contained freshly ingested blood. Therefore it seems that the larvae, as their mouthparts would suggest, suck liquid food from the tissues of the host. When the larva first becomes attached it may sever a blood capillary of the host and the released blood would be ingested. When the tissue response is well established the only fluid available would be the tissue exudate which must be the main food of the larva. Roy (1937) was of the opinion that the larvae fed on the stomach contents of the host, but this is unlikely as, when smeared and stained with Giemsa solution, the mid-gut contents were almost entirely free of bacteria, whereas the stomach contents of the host have an extremely rich bacterial flora. Vogelsang, Gallo & Mayaudon (1955) found a marked bactericidal activity, in the mid-gut contents of larvae of *G. intestinalis*, against some common pathogenic bacteria. The mid-gut contents were therefore investigated for the presence of any activity towards the bacteria of the horse's stomach, but no such activity was found.

PREPARATION OF ENZYME MATERIALS

Wigglesworth (1927) found that some carbohydrase activity was strongly inhibited in enzyme preparations made up in glycerol, so, for all experiments, a fresh mid-gut or salivary gland emulsion was prepared with distilled water in a small homogenizer. The amount of emulsion per experimental tube was generally 0·1 ml. and this contained the mid-gut from one larva or one pair of salivary glands. For the mid-gut carbohydrase experiments an incubation time of 10 min. at 30° C. was sufficient, but for the salivary gland carbohydrases, mid-gut proteinase and the lipase the tubes were shaken at 30° C. for at least 24 hr. Control tubes were included in all experiments with heat-inactivated enzyme material. Bacterial activity

was prevented by the addition of small amounts of toluol. Throughout the investigations the buffer solutions were those of Clark and Lubs as given in Cole (1955).

EXPERIMENTAL RESULTS

pH *of the contents of the larval gut.* The hydrogen-ion concentration in the mid-gut was roughly determined by means of the B.D.H. Capillator. The mid-gut was dissected out on to a waxed slide and the contents drawn up with a little neutral distilled water in a micropipette. They were then mixed with a little B.D.H. indicator and the pH read off from the capillator. The pH was found to vary very little from between pH 7·1 and 7·3, which is close to the figure of pH 7·4 given by Roy (1937).

CARBOHYDRASES

The effect of mid-gut and salivary-gland emulsions on various carbohydrate substrates has been studied. Preliminary experiments showed that a mixture of starch solution and mid-gut emulsion soon lost the ability to give the characteristic blue colour with a solution of iodine and gained the power of reducing Benedict's solution. The osazone test also showed the formation of a heavy precipitate of glucosazone and maltosazone crystals.

The saccharogenic properties were then studied in more detail, using the method of Hagedorn and Jensen, (Cole, 1955), to compare activity in terms of the production of glucose equivalent reduction. The experiments were conducted as follows: to each of a series of test tubes were added 0·25 ml. 2% substrate solution (in the case of maltose and lactose 0·5% solutions were used), 0·25 ml. buffer solution, 0·1 ml. fresh mid-gut or salivary-gland emulsion and a drop of toluol. The controls were heated in a water-bath to 100° C. before the addition of the enzyme material and maintained at that temperature for a few minutes to inactivate the enzymes. The tubes were shaken at 30° C. for periods of 10–45 min. Longer experiments showed that after the first hour the rate of reaction declined steadily. The reaction was stopped by placing the tubes in a water-bath at 100° C., after which 0·1 ml. samples were withdrawn and the amounts of glucose equivalent determined. It was not found necessary to precipitate and filter off the protein.

Amylase in the mid-gut. Table 1 shows the results, in terms of mg. glucose equivalent per 100 ml., of a typical experiment after a 10 min. incubation of starch-mid-gut reaction mixtures at different values of hydrogen-ion concentration. In Fig. 1 results of similar experiments are expressed in terms of the relative reaction velocities of the amylase at different hydrogen-ion concentrations. The peak of activity is seen to be in the region of pH 6.

Mammalian and insect amylases have been shown to be activated by the addition of certain cations, particularly Cl', to the reaction mixture, and Table 1 also shows the increased production of glucose equivalents after the addition of 0·1 ml. 0·5 M-NaCl. Fig. 2 is a graph of amylase activity with and without NaCl, and shows that while the peak has remained at pH 6 it has broadened considerably with a greatly increased activity, particularly on the alkaline side of the peak.

The amyloclastic properties of the amylase were also well marked and with

a reaction mixture of 5 ml. 1 % starch and 0·5 ml. emulsion containing the mid-guts of 10 larvae the following results were obtained. Test samples quickly lost the ability to produce a blue colour with iodine solution, becoming red after 20 min. and, after 30 min., no colour change was detectable.

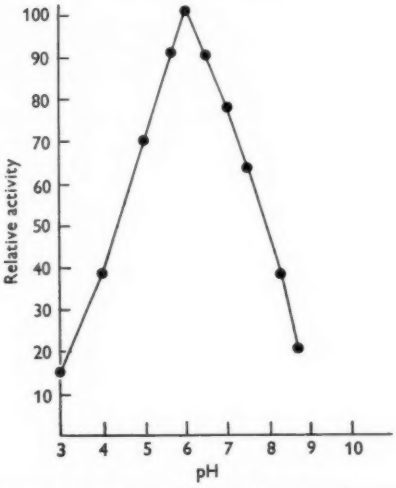


Fig. 1. The relative activity of the mid-gut amylase at different hydrogen-ion concentrations.

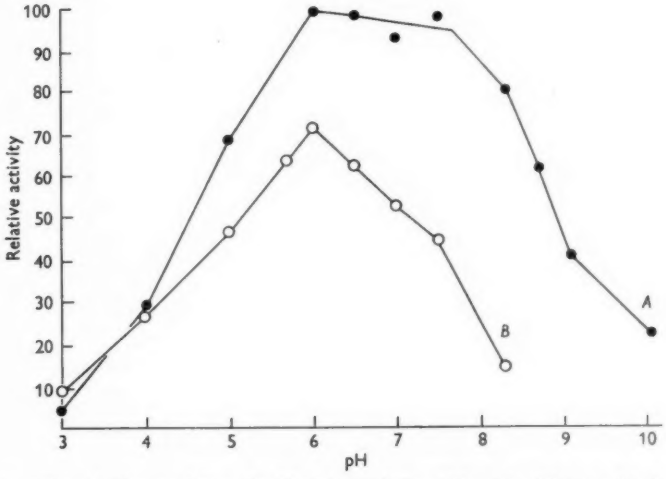


Fig. 2. The relative activity of mid-gut amylase, with and without added Cl′, at different hydrogen-ion concentrations. *A*, with Cl′; *B*, without Cl′.

Amylase in the salivary glands. The salivary glands were dissected out and after thorough washing in saline and distilled water, an emulsion prepared. Because of the small amount of material involved it was necessary to incubate the reaction

mixture for 24 hr. Thus, after an incubation period of 24 hr., a reaction mixture containing one pair of salivary glands would have produced 272 mg./100 ml., while after only 10 min. incubation a reaction mixture containing one mid-gut would have produced 60 mg./100 ml. The optimum pH for the enzyme was again at pH 6, the appearance of the peak being similar to that of the mid-gut amylase.

TABLE 1. *The results of a typical experiment with mid-gut amylase incubated with a starch solution for* 10 *min. and of a similar experiment after the addition of* 0·1 *ml.* 0·5 M *solution of* NaCl

	mg./100 ml. glucose equivalent	
pH	Without NaCl	With NaCl
3·0	9	6
4·0	23	25
5·0	40	70
5·7	47	—
6·0	56	80
6·5	43	75
7·0	40	71
7·5	37	68
8·3	12	63

Amylase in the haemolymph. As first mentioned by von Kemnitz (1914), a powerful amylase is present in the haemolymph of the larvae of *Gasterophilus*. Although it was felt that the mid-guts and salivary glands had been sufficiently well washed to have removed any traces of haemolymph amylase there was a possibility in the case of low activity shown by the salivary gland amylase that

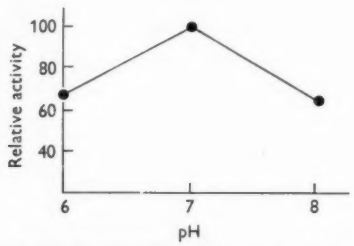


Fig. 3. The relative activity of the haemolymph amylase at different hydrogen-ion concentrations.

some contamination had occurred. A study of the relative activity of the haemolymph amylase at different hydrogen-ion concentrations was made and it was found that maximum activity took place at pH 7, and from Fig. 3 it can be seen that at pH 6 the relative activity is only 70 % of the maximum. The amylase of the haemolymph is therefore a different enzyme from that found in the mid-gut or the salivary glands and experiments involving the latter had not been contaminated with haemolymph.

Maltase in the mid-gut. With maltose as a substrate, enzyme activity was detected by measuring the increase in glucose equivalent reduction by Hagedorn

and Jensen's method and by the demonstration of glucosazone crystals. Measurement of maltase activity over a range of hydrogen-ion concentrations showed that its maximum activity was over a wide peak at pH 6.

Invertase in the mid-gut. Preliminary testing with Benedict's solution, after incubation of a 2 % sucrose-gut emulsion mixture, gave an intense positive reaction; and an osazone test showed that glucose was being produced. A study of the reaction velocities at different hydrogen-ion concentrations again showed the peak to be at pH 6.

Lactase in the mid-gut. Osazone tests for the production of glucose after the incubation of a gut emulsion with a 0·5 % lactose solution were negative. Although this by itself is not conclusive, it was not possible to demonstrate any increase in the reduction equivalent of the reaction mixture and it was therefore decided that a lactase was not present.

Saccharases in the salivary glands. As with the investigation of salivary-gland amylase, it was necessary to incubate the reaction mixture for 24 hr. in the tests for salivary-gland saccharase. Reaction mixtures were made up at pH 6, which was assumed to be the optimum for any saccharase present, with 2 % sucrose and 0·5 % maltose as substrates. With both substrates an increase in glucose equivalent reduction occurred, demonstrating the presence of an invertase and a maltase.

PROTEINASES

Proteinase activity was mainly investigated by the formol titration technique of Sorensen as modified by Blacklock, Gordon & Fine (1930), but some experiments were carried out by the method of Anson & Mirsky (1933), adapted for use with small amounts of material. Estimations of activity in terms of an increase in 'formol acidity' were made from 0·1 ml. samples taken from a reaction mixture of 0·5 ml. 4 % casein or gelatine, 0·5 ml. buffer solution, 0·1 ml. gut emulsion and a few drops of toluol, incubated at 30° C. for 24–48 hr. The control tubes were heated to 100° C. in a water-bath before the addition of the enzyme material and maintained at that temperature for a short time to inactivate the enzymes.

Proteinase in the mid-gut. Preliminary tests of proteinase activity in terms of the amount of dye released from particles of fibrin coloured with congo red showed that there was no activity in the region of pH 2, from a pepsin-like enzyme, and that the maximum activity occurred around pH 8. Table 2 shows the amounts of 'formol acidity' produced in a typical experiment, with either casein or gelatine as substrate, at different hydrogen-ion concentrations. In the case of gelatine the relative activity curve (Fig. 4) has a double peak at pH 7·5 and 8·7, while for casein the peak is regular and lies at pH 8·3.

Peptidases in the mid-gut. Peptidase activity was studied by the same method as for the proteinase. The substrates for the reaction mixtures were 0·2 M solutions of glycyl-glycine, glycyl-L-proline, DL-leucyl-glycyl-glycine, chloracetyl tyrosine and a saturated solution of DL-leucyl-glycine. With glycyl-glycine the pH activity curve repeats the double peak at pH 7·5 and 8·7 seen with gelatine (Fig. 5). The reduction in activity at pH 8·3 was, however, relatively slight, compared with that with the

gelatine. The pH activity curve with DL-leucyl-glycyl-glycine showed a single peak at pH 7·5.

With the sparingly soluble DL-leucyl-glycine the results obtained were inconclusive and no clear di-peptidase activity could be shown. The completely negative results obtained with glycyl-L-proline and chloracetyl tyrosine indicated the absence of a gut prolidase and of a carboxy di-peptidase.

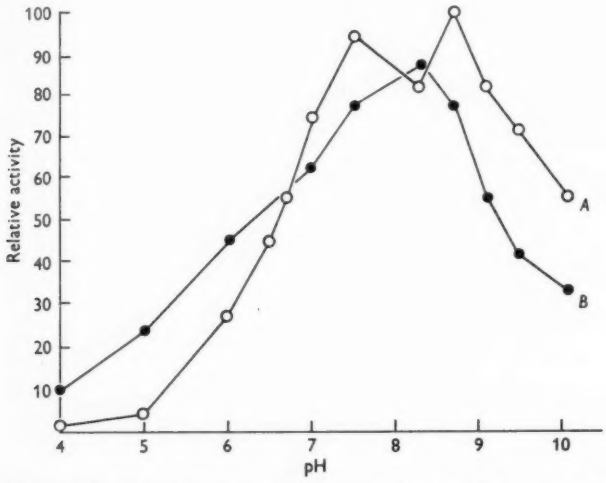


Fig. 4. The relative activity of the mid-gut proteinase with casein and gelatine. *A*, gelatine; *B*, casein.

TABLE 2. *The results of typical experiments, with mid-gut proteinase incubated with casein and gelatine for 24 hr., in terms of increase in 'formol acidity'*

	ml. N/100 NaOH			ml. N/100 NaOH	
pH	Casein	Gelatine	pH	Casein	Gelatine
4·0	0·12	0	7·5	0·90	1·16
5·0	0·29	0·04	8·3	1·08	0·95
6·0	0·55	0·30	8·7	0·85	1·25
6·5	—	0·52	9·1	0·65	0·93
6·7	—	0·65	9·5	0·47	0·82
7·0	0·77	0·89	10·1	0·35	0·68

Proteinase in the salivary glands. Roy (1937) found that salivary gland emulsions were capable of clotting calcified milk and yet were unable to produce any detectable digestion of casein or gelatine solutions. In the present work that observation has been confirmed, but without the addition of any further information.

LIPASE AND ESTERASE

The method of this investigation was adapted for use with small amounts of material from that described by Fodor (1948). Gum arabic is used as an emulsifying agent in the preparation of a 1 millimole/5 ml. distilled-water emulsion of the sub-

strate in order to give a 1 millimole/10 ml. reaction mixture on the addition of buffer solution. A reaction mixture consisted of 0·25 ml. substrate emulsion, 0·25 ml. buffer solution and 0·1 ml. mid-gut emulsion with a few drops of toluol. The mixture was then shaken at 30° C. for 24–48 hr. Control tubes were heated to

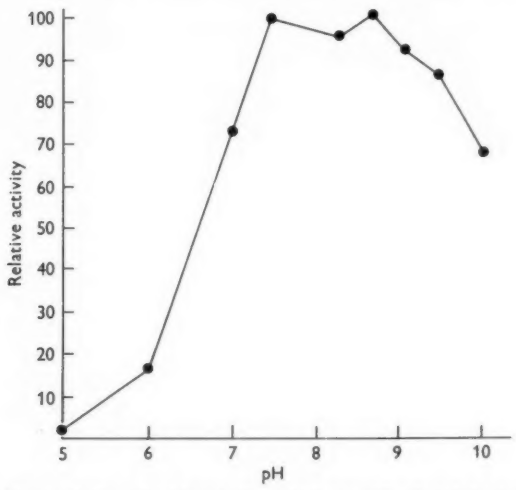


Fig. 5. The relative activity of mid-gut di-peptidase with glycylglycine as a substrate.

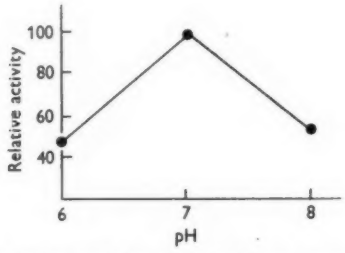


Fig. 6. The relative activity of the mid-gut lipase at different hydrogen-ion concentrations.

100° C. in order to inactivate the enzyme. The substrates investigated were olive oil, tributyrin and ethyl butyrate. At the conclusion of the incubation period 0·2 ml. samples were removed into 5 ml. distilled water and titrated with N/100 NaOH, using phenolphthalein as an indicator. The difference between the control and experimental tubes was due to the production of fatty acids during digestion. A series of determinations using the 'formol titration' method showed that differences due to autolytic digestion of proteins were negligible. Unfortunately, positive results were obtained only with tributyrin as a substrate. However, the pH activity curve with tributyrin shows a clear peak at pH 7·0 (Fig. 6).

Lipase of the haemolymph. Reaction mixtures were also made up containing haemolymph, tributyrin and buffer solution. The haemolymph was shown to contain a lipase similar to that of the gut in its pH activity curve.

SALIVARY GLAND ANTICOAGULIN

The presence of an anticoagulin in the secretion of the salivary gland, referred to by Dinelescu (1932), was confirmed using the method of Yorke & Macfie (1924). A pair of glands was macerated in 0·035 ml. saline and mixed with 0·070 ml. of fresh blood. The mixture was drawn up into a wide bore capillary tube and incubated at 25° C. A control tube, of blood and saline only, coagulated in 4 min., while, after 18 hr., the blood in the experimental tube was still perfectly liquid.

A similar experiment using a gut-saline emulsion also prevented any coagulation and after 18 hr. some haemolysis and digestion had occurred.

DISCUSSION

Although the diet of larvae of *G. intestinalis* would seem to be highly specialized and to consist almost entirely of protein, the complement of digestive enzymes is quite unspecialized in its broad outline, and includes highly active carbohydrases.

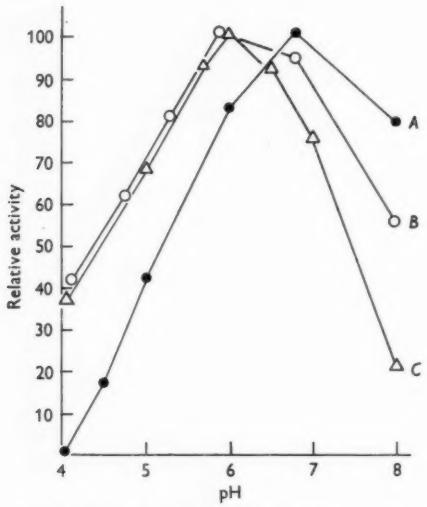


Fig. 7. The relative activity of ptyalin (*A*), *Blatella* amylase (*B*), and *Gasterophilus* amylase (*C*), at different hydrogen-ion concentrations. (Partly after Wigglesworth, 1927.)

The presence of a maltase and an invertase in the secretion of the salivary glands is particularly striking. Wigglesworth (1927) had found an invertase in *Blatella*, but not in *Periplaneta*, although the complete absence of saccharases in the salivary glands of other insects, until Evans (1956) found them in those of *Calliphora*, had suggested an analogy with the secretion of the mammalian salivary glands.

The pH activity curve of the mid-gut amylase shows a great similarity with that, given by Wigglesworth, for the mid-gut amylase of the cockroach, except that it is less tolerant of increasing alkalinity. In Fig. 7 the amylase of the cockroach, that of *Gasterophilus* and human ptyalin are compared.

The broadening of the peak of amylase activity after the addition of Cl′ ions to the reaction mixture is a common occurrence, and here it has the effect of raising the activity in the normal physiological range from 70 % of the maximum to 95 %.

A comparison of the proteinase activity curves with those of the cockroach and *Glossina*, given by Wigglesworth (1928, 1929), is complicated by the double peak obtained when gelatine is the substrate, but the curve for casein appears to have closer affinities with the curve for mammalian trypsin, as can be seen from Fig. 8. The curve for a polypeptidase and DL-leucyl-glycyl-glycine follows the insect curve closely from pH 5 to 7·5, but then falls off rapidly as the alkalinity increases. However, the results indicate that there are a variety of different enzymes involved in the splitting of proteins to amino acids and that any activity curve obtained from a crude enzyme preparation must represent an over simplification.

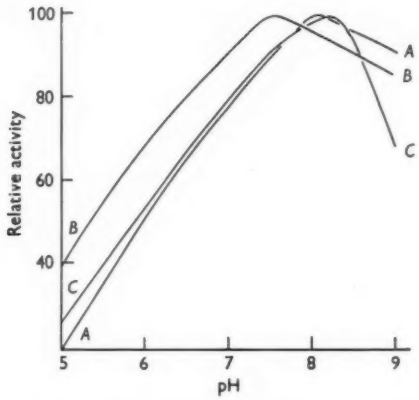


Fig. 8. The relative activity of mammalian trypsin (*A*), cockroach and *Glossina* proteinase (*B*), and *Gasterophilus* proteinase (*C*), at different hydrogen-ion concentrations. (Partly after Wigglesworth, 1929.)

The technique used in the lipase determinations was adequate only for demonstrating the trends of activity and the negative results with olive oil and ethyl butyrate are probably not significant. This is so particularly in the case of ethyl butyrate, as Fodor (1948*a*) mentions that gum arabic causes an inhibition of from 60 to 100 % with some insect esterases. Fodor (1948*b*), working with the gut lipase of the silkworm larva (*Bombyx mori*), also found that, like *Gasterophilus*, the maximum activity with a substrate of tributyrin was at pH 6·8–7·0.

Arvy & Gabe (1946) showed that the presence of an amylase or a lipase, or both, in the haemolymph of insects is quite common. Both of these enzymes were found in *Gasterophilus*, and in the case of amylase the enzyme was clearly of a different nature to the one found in the gut.

The conclusion to be drawn from these results is that the highly specialized diet of an endoparasitic insect larva is not to be correlated with any reduction in the normal insect digestive enzyme complement.

SUMMARY

1. Evidence is produced to show that the larvae of *Gasterophilus intestinalis* are occasional blood feeders but normally feed on tissue exudate.

2. The demonstration, by Roy (1937), of the following enzymes has been confirmed and curves of their activity at different hydrogen-ion concentrations have been constructed: mid-gut amylase, proteinase and lipase.

3. The following enzymes have been demonstrated and curves of their activity at different hydrogen-ion concentrations have been obtained: maltase, invertase, polypeptidase and di-peptidase of the mid-gut and amylase, maltase and invertase of the salivary glands.

4. The haemolymph was shown to contain a lipase as well as an amylase and an anticoagulin.

Thanks are due to Dr P. Tate for his advice and encouragement throughout the investigation and in the preparation of the manuscript.

REFERENCES

ANSON, M. L. & MIRSKY, A. E. (1933). The estimation of trypsin with haemoglobin. *J. Gen. Physiol.* **17**, 151–7.

ARVY, L. & GABE, M. (1946). Identification des diastases sanguine chez quelques insectes. *C.R. Soc. Biol. Paris*, **140**, 757–8.

BLACKLOCK, D. B., GORDON, R. M. & FINE, J. (1930). Metazoan immunity: a report on recent investigations. *Ann. Trop. Med. Parasit.* **24**, 5–54.

COLE, S. W. (1955). *Practical Physiological Chemistry*, 10th ed. Cambridge: Heffer.

DAY, M. F. & WATERHOUSE, D. F. (1953). *Insect Physiology*, ed. by K. D. Roeder. New York: John Wiley.

DINELESCU, G. (1932). Recherches sur la biologie des Gastrophiles. *Ann. Sci. Nat. Zool.* (10), **15**, 1–184.

EVANS, W. A. L. (1956). Studies on the digestive enzymes of the blow-fly, *Calliphora erythrocephala*. I. The carbohydrases. *Exp. Parasitol.* **5**, 191–206.

FODOR, P. J. (1948*a*). Studies on the nature of insect lipases and esterases. II. The lipases and esterases of the grasshopper. *Enzymologia*, **12**, 333–42.

FODOR, P. J. (1948*b*). The specificity of the lipases and esterases contained in the larva of the silkworm (*Bombyx mori*). *Enzymologia*, **13**, 66–72.

FRAENKEL, G. (1940). Utilisation and digestion of carbohydrates by the adult blow-fly. *J. Exp. Biol.* **17**, 18–29.

HOBSON, R. P. (1931). Studies on the nutrition of blow-fly larvae. *J. Exp. Biol.* **8**, 109–23.

KEMNITZ, G. A. VON. (1914). Untersuchungen über Stoffbestand und Stoffwechsel der Larven von *Gastrophilus equi*. *Verh. dtsch. Zool. Ges.* **24**, 294–307.

ROY, D. N. (1937). The physiology of digestion in larvae of *Gastrophilus equi*. *Parasitology*, **29**, 150–62.

SIMMONS, S. W. (1939). Digestive enzymes of the cattle-grub, *Hypoderma lineatum*. *Ann. Ent. Soc. Amer.* **32**, 621–7.

VOGELSANG, E. G., GALLO, P. & MAYAUDON, T. H. (1955). Elementos bacteriostaticos en las larvas de *Gastrophilus* (nota previa). *Rev. Med. Vet. Parasit. Caracas*, **14**, 41–4.

WIGGLESWORTH, V. B. (1927). Digestion in the cockroach. II. The digestion of carbohydrates. *Biochem. J.* **21**, 791–811.

WIGGLESWORTH, V. B. (1928). Digestion in the cockroach. III. The digestion of proteins and fats. *Biochem. J.* **22**, 150–61.

WIGGLESWORTH, V. B. (1929). Digestion in the tse-tse: a study of structure and function. *Parasitology*, **21**, 288–322.

WIGGLESWORTH, V. B. (1931). Digestion in *Chrysops silacea* Aust. (Diptera, Tabanidae). *Parasitology*, **23**, 73–8.

YORKE, W. & MACFIE, J. W. S. (1924). The action of the salivary-gland secretion of mosquitoes and of *Glossina tachinoides* on human blood. *Ann. Trop. Med. Parasit.* **18**, 103–8.

A STUDY OF THE EARLY STAGES OF INFECTION WITH THE TIPULA IRIDESCENT VIRUS

By KENNETH M. SMITH

Virus Research Unit, Agricultural Research Council, Cambridge

(With Plates XIV–XVII)

INTRODUCTION

The Tipula Iridescent Virus (TIV) occurs in the larva of a fly, *Tipula paludosa*, the crane fly or daddy-long-legs. A similar virus has not been observed in the larvae of any other species of *Tipula* or indeed of any other insect.

The primary site of development of the virus is in the cytoplasm of the fatbody cells; it has never been observed in the cell nucleus (Williams & Smith, 1957). It is a comparatively large virus measuring 130 mμ in diameter and is extremely uniform in size and shape. The topography of the virus particle has recently been elucidated, and it has been shown to be an icosahedron, having 20 sides (Smith & Williams, 1958; Williams & Smith, 1958). It is an interesting fact that up to the present all insect viruses that develop in the cell cytoplasm have proved to be nearly spherical or polyhedral in shape, whereas all those so far described with nuclear development have been rod-shaped.

The Tipula Iridescent Virus occurs in enormous quantities in the fatbody of the infected insect, and no less than 25% of the dry weight of the larva is converted into virus (Williams & Smith, 1957). About 15% of the virus particle is deoxyribose nucleic acid (J. D. Smith, unpublished).

The crystallization of the virus in the insect, together with its uniformity of size, have resulted in the peculiar optical properties from which the name of the virus has been derived. When the virus has been concentrated by centrifuging at 12,000 r.p.m. for 40 min., the resulting pellet shows an intense iridescence (Smith & Williams, 1958). Indeed an infected larva can be immediately recognized by its violet coloration caused by the optical effect of the micro-crystals of virus in the fatbody of the living insect.

In an earlier study of this virus with the electron microscope by means of ultra-thin sections (Smith, 1956), it was shown that each virus particle was surrounded by a membrane and was often hexagonal in section. Moreover, numerous apparently empty membranes of approximately the same size as the virus particle frequently occurred. The present study is concerned with the significance of these empty membranes.

MATERIAL AND METHODS

Larvae of *T. paludosa* were used in varying stages of infection, attention being paid mainly to the early stages. Such stages were not easy to find since by the time the violet colour of the fatbody was visible, which is the earliest diagnostic

character, the disease was already far advanced. Tests of larvae were therefore made at intervals after inoculation; this was a laborious and time-consuming operation since the fatbody from each larva tested had to be fixed, sectioned and examined in the electron microscope. Apart from the violet coloration of the fatbody, which develops at too late a stage for the purpose of this study, there is no method so far as the writer is aware of diagnosing an early infection other than by electron microscopy.

The fatbody was dissected out of each infected larva and fixed in 2% buffered osmic acid for 2 hr. It was then dehydrated in ethanol in 10% steps and embedded in a mixture consisting of 8 parts butyl- to 2 parts methyl-methacrylate.

Sections were cut on a Cook and Perkins ultra-microtome using glass knives, and mounted on formvar- or carbon-covered grids. The electron micrographs were taken on a Siemens Elmiskop I microscope at 60 kV. Fixation of the fatbody in osmic acid renders the contained fat globules extremely hard with a consequent tendency for the sections to be compressed. In this connexion Sotelo's (1957) chloroform-flattening technique was found helpful.

RESULTS

In ultra-thin sections of fatbody from a normal larva of *T. paludosa* the endoplasmic reticulum is seen clearly enough with numerous fat globules rendered black by osmic fixation. The first obvious point of difference between the normal and diseased fatbody is the gradual disappearance of the endoplasmic reticulum and in the immediate vicinity of the particles it is completely absent (Pl. XVII, fig. 1).

In sections through the fatbody at an early stage of infection many empty membranes, often more numerous than the virus particles themselves, can be observed (Pl. XIV). These membranes are approximately the same size and shape as the virus particles which appear black in the micrograph. They have not been observed in sections of normal fatbody and apparently do not occur in the late stages of the disease (compare Pl. XVII, fig. 2).

In addition to the apparently empty membranes, others occur with a central particle which may be spherical or thread-like. The size of the central particle varies from a barely visible speck to the stage where it almost fills the membrane. These different forms are shown in Pl. XIV and at higher magnification in Pl. XVI.

An interesting point, which may have no significance, is the remarkable resemblance between the empty virus membranes and the oval forms of the endoplasmic reticulum. Indeed, in some cases it is difficult to differentiate between the two on the electron microscope. This is well shown in the upper part of Pl. XV.

DISCUSSION

There seem to be three possible explanations for the occurrence of the empty virus membranes: first, they could be incomplete or malformed virus particles; secondly, they might be stages in the breakdown or dissolution of the virus; and thirdly, they could be stages in the development or multiplication of the virus. From a careful study of many sections of fatbody from different larvae, the first two

possibilities seem unlikely. The empty membranes are far too numerous, and the fact that they appear mainly in the early stages of infection and have not been observed in the late stages (Pl. XVII, fig. 2) is strongly in favour of the suggestion that they are developmental forms. In addition, there seems no particular reason for supposing that they are breakdown stages of the virus.

If we assume then that these empty membranes are in fact the first stages of virus multiplication, it is an interesting question as to how the deoxyribose nucleic acid enters the apparently empty membrane, always supposing, of course, that the darkly staining centre of the virus particle contains the DNA and that the latter does not make up the membrane itself.

Somewhat similar empty or partly empty membranes have been observed in one or two other virus diseases. Fawcett (1956), working on the Lucké renal adenocarcinoma of the leopard frog, describes numerous empty-appearing vesicles in the nucleus. A few of these contain a dense inner body and he suggests that they may be immature virus particles.

The apparent mode of development of TIV differs from that described by Morgan, Ellison, Rose & Moore (1954), for the virus of herpes simplex. Here it is suggested that a 'primary body' forms first which then becomes enclosed in a single outer membrane. Upon release from the nucleus into the cytoplasm these particles appear to acquire a second outer membrane and presumably represent the mature virus. In the TIV the order of development seems different in that the membrane develops first and the 'primary body' appears later inside the membrane. In what are thought to be the very early stages the TIV primary body seems to have a thread-like or sometimes spherical appearance.

There is only one limiting membrane to the virus particle.

SUMMARY

Ultra-thin sections and electron microscopy have been used in a study of the empty membranes which occur in the fatbody cells of the larva of *T. paludosa* infected with the Tipula Iridescent Virus (TIV).

It has been observed that these empty membranes are most numerous in the early stages of infection. Later an apparent 'primary body', which may be thread-like in its initial stages, develops in the centre of the membrane. This primary body seems to increase in size until the membrane is filled, and the various steps in this process can easily be seen.

It is suggested that the formation of the empty membranes and the apparent gradual development of their contents are the means by which the virus multiplies.

The remarkable resemblance between the empty virus membranes and the endoplasmic reticulum is pointed out.

The expenses of the Plates were defrayed by a grant from the Agricultural Research Council. Acknowledgement is due to Mr G. J. Hills for taking the electron micrographs.

REFERENCES

FAWCETT, D. W. (1956). *J. Biophysic. Biochem. Cytol.* **2**, 725–41.
MORGAN, C., ELLISON, S. A., ROSE, H. M. & MOORE, D. H. (1954). *J. Exp. Med.* **100**, 195–202.
SMITH, K. M. (1956). *J. Biophysic. Biochem. Cytol.* **2**, 301–6.
SMITH, K. M. & WILLIAMS, R. C. (1958). *Endeavour*, **17**, 12–21.
SOTELO, J. R. (1957). *Exp. Cell. Res.* **13**, 599–601.
WILLIAMS, R. C. & SMITH, K. M. (1957). *Nature, Lond.*, **179**, 119–20.
WILLIAMS, R. C. & SMITH, K. M. (1958). *Biochim. biophys. Acta* (in the Press).

EXPLANATION OF PLATES XIV–XVII

PLATE XIV

Ultra-thin section through the fatbody from a larva of *T. paludosa* in an early stage of infection: note the numerous empty membranes and central bodies of various sizes indicated by arrows. × 40,000.

PLATE XV

Similar to Pl. XIV but showing the resemblance of the empty virus membranes to the endoplasmic reticulum in the upper part of the micrograph. × 40,000.

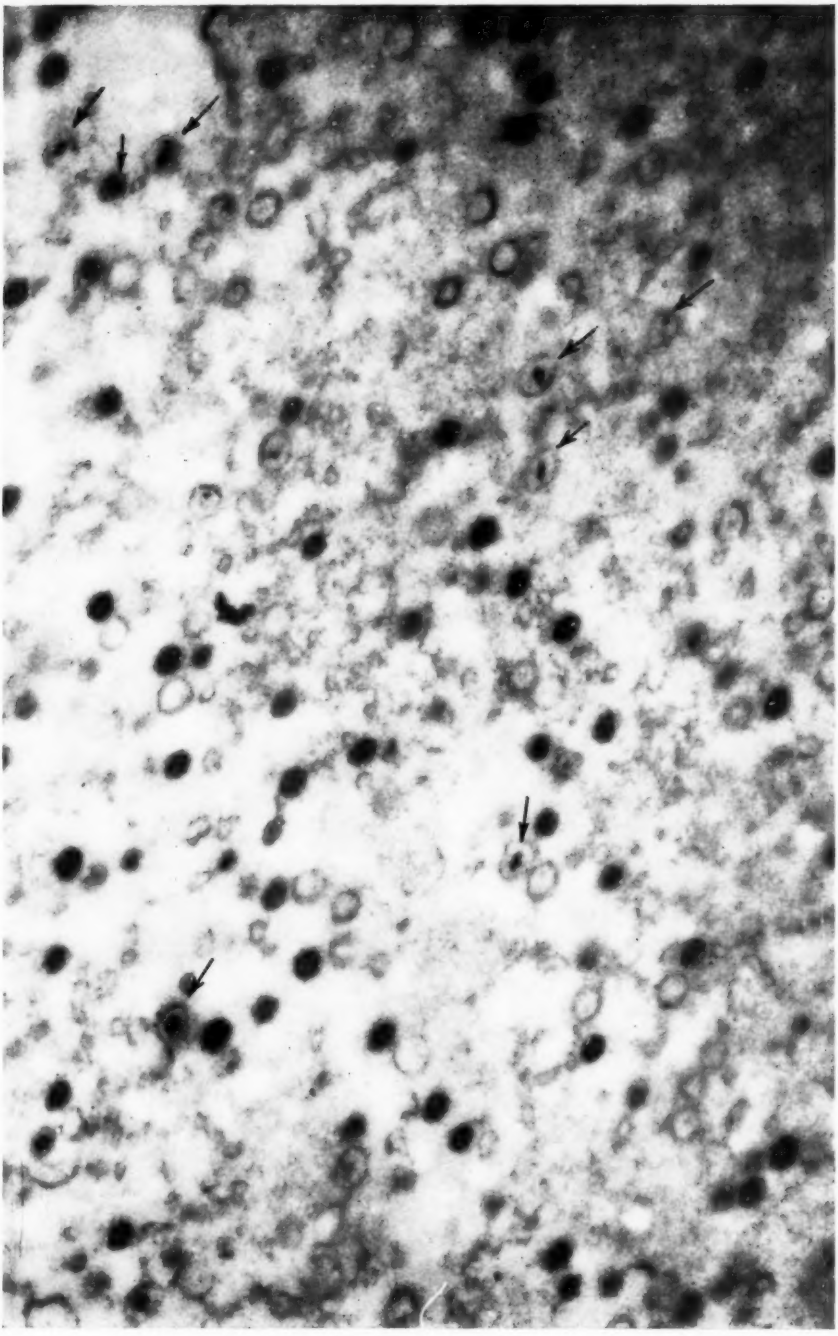
PLATE XVI

Ultra-thin sections at high magnification of TIV particles showing the apparent developmental stages. × 93,000.

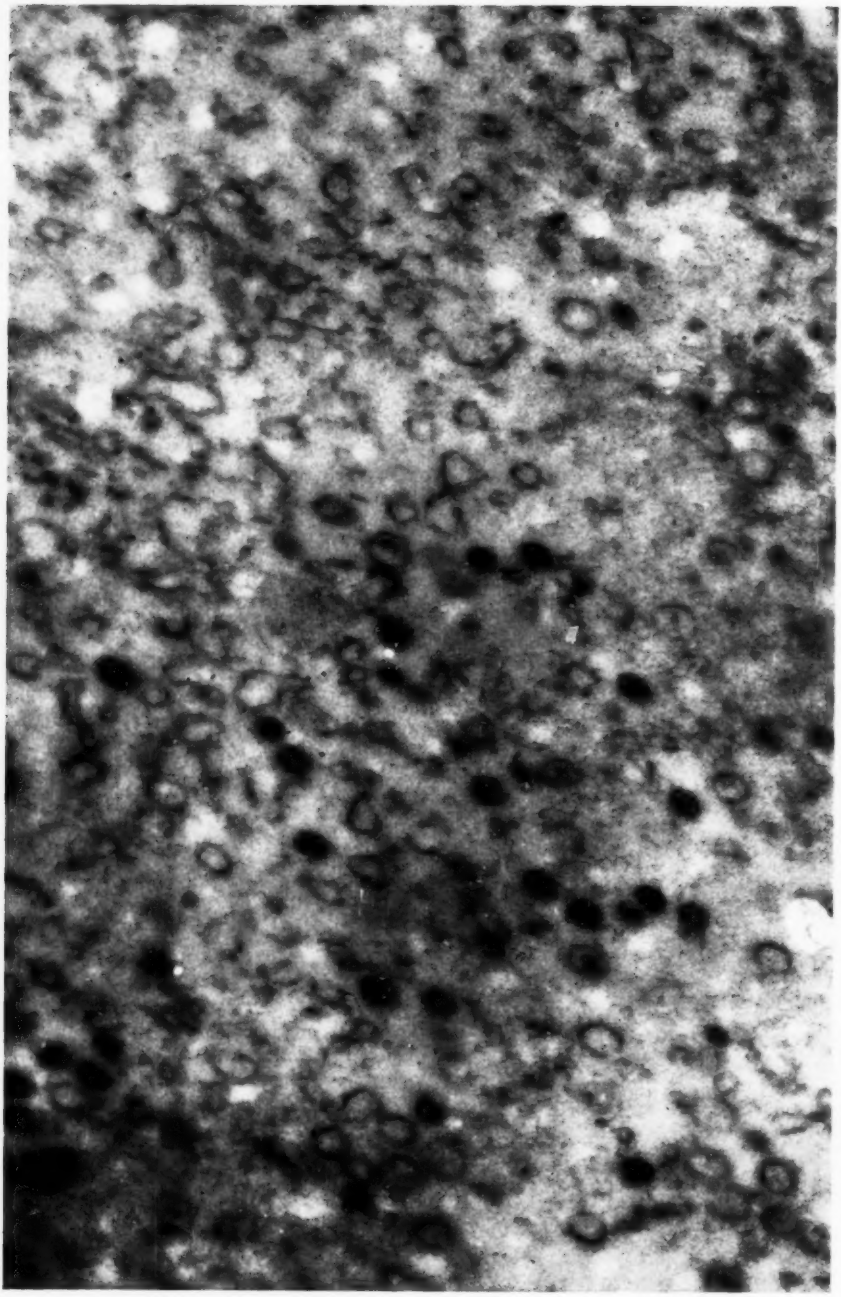
PLATE XVII

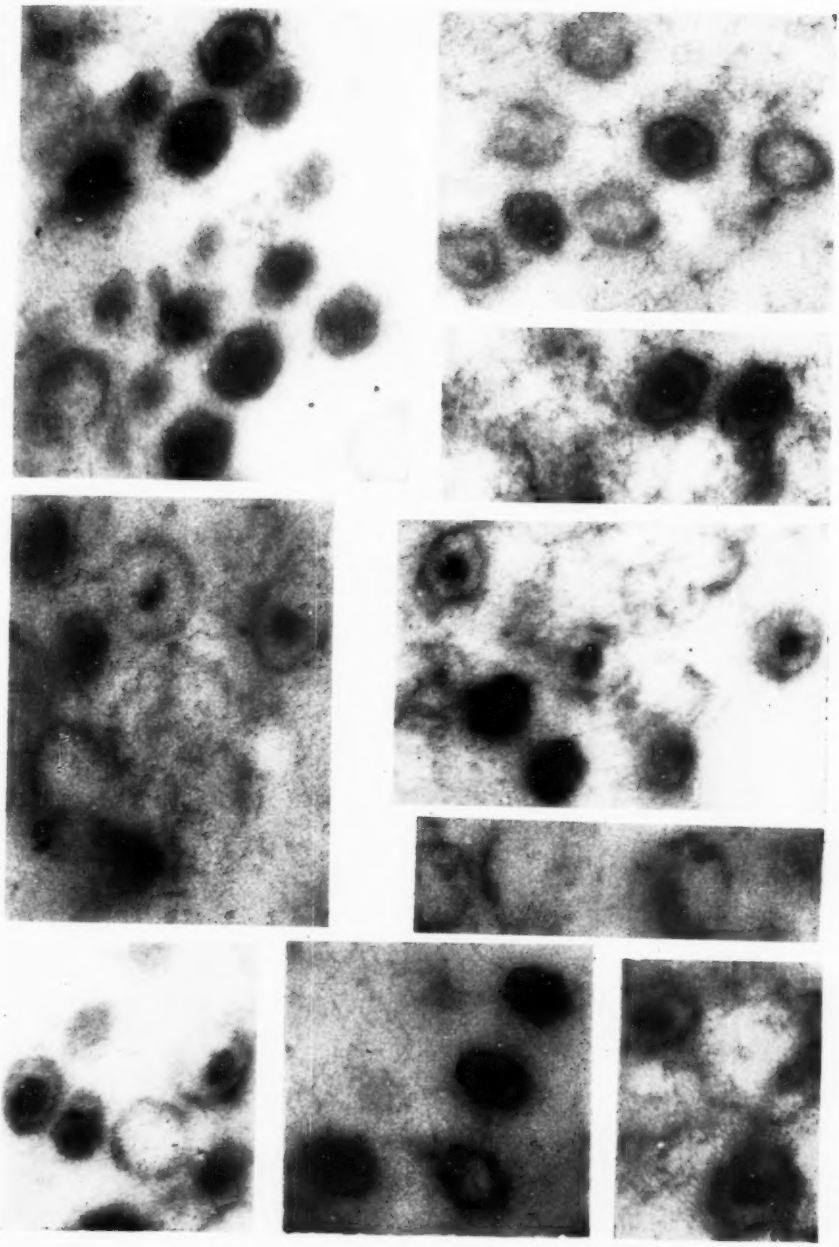
Fig. 1. Ultra-thin section of infected fatbody showing the disappearance of the endoplasmic reticulum in the presence of the virus. × 36,000.

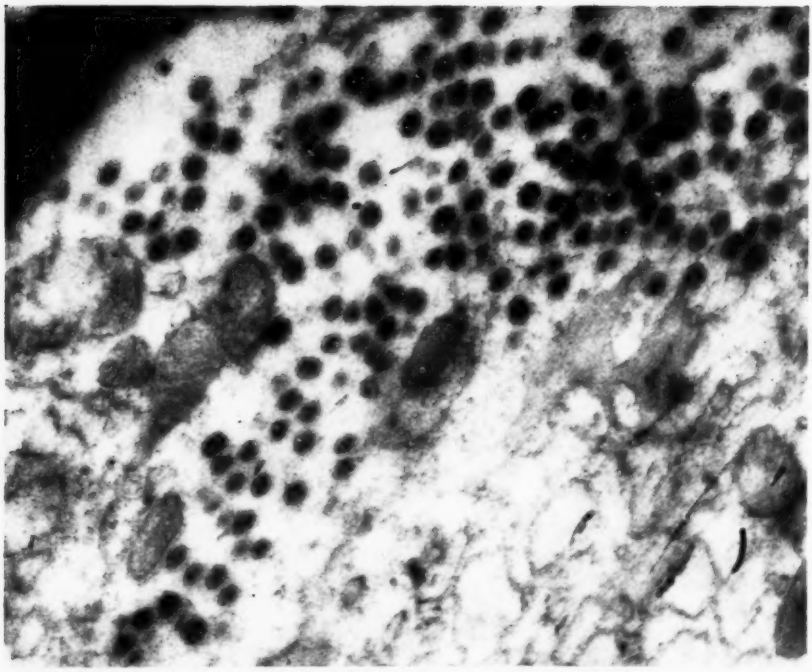
Fig. 2. Ultra-thin section of infected fatbody in a late stage of infection; note the numerous virus particles beginning to line up to form a micro-crystal and the absence of empty membranes. × 18,000.

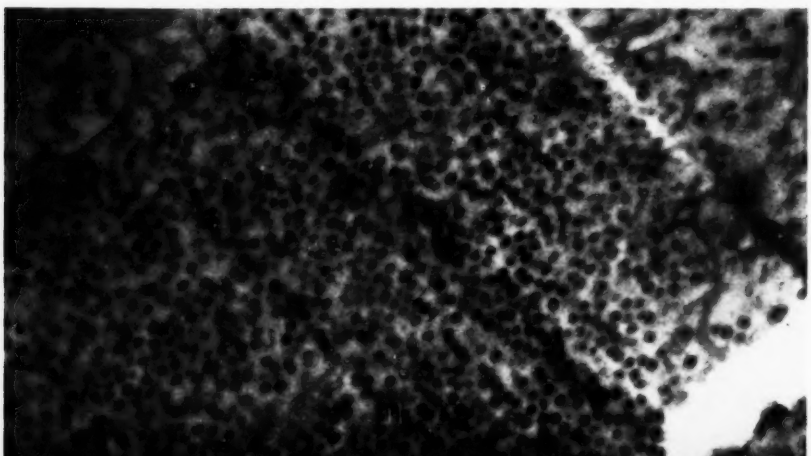


(*Facing p.* 462)









THE EFFECT OF IMMUNE SERA ON THE RESPIRATION OF *TRYPANOSOMA BRUCEI IN VITRO*

By JUNE P. THURSTON

*Molteno Institute, University of Cambridge**

(With 2 Figures in the Text)

INTRODUCTION

Desowitz (1956) showed that serum from N'Dama cattle infected with *Trypanosoma vivax* reduced the oxygen uptake of *T. vivax in vitro* and caused lysis of the trypanosomes. He suggested that the reduction in oxygen uptake might be used to measure the titre of antibody in the serum.

Soltys (1957) demonstrated the effect of neutralizing antibodies against *T. brucei*. During these experiments he observed that high concentrations of immune sera reduced the motility of trypanosomes within 1 hr., whereas trypanosomes suspended in antisera diluted by more than 1/10 were still active after 1 hr., but were not infective when inoculated into mice.

It was suggested that I should measure the oxygen uptake of *T. brucei* in lower and higher dilutions of antisera, using the techniques evolved earlier for measuring the oxygen uptake of *T. equiperdum* (Thurston, 1958*a*, *b*). The results of these experiments are reported in this paper.

MATERIALS AND METHODS

The strain of *T. brucei* 'O' and immune sera were supplied by Dr Soltys. The strain of *T. brucei* 'O' since its isolation in East Africa had been passaged only through mice and was sensitive to antibodies. The sera were collected from rabbits four weeks after inoculation with *T. brucei* 'O'. Four sera were used in these experiments, with titre of neutralizing antibody varying between 1/80 to 1/320. All sera were kept in deep freeze at $-35°$ C. from the time of preparation until they were used.

The serum R 93 was prepared $2\frac{1}{2}$ months previously and had the titre 1/160.

The serum N 93 was prepared $5\frac{1}{2}$ months previously and had the titre 1/80.

The serum M 5 was prepared 1 month previously and had the titre 1/160.

The serum OR 17 was fresh antiserum and had the titre 1/320.

Normal rabbit serum was also tested in each series of experiments as a control.

During the present experiments, rats were infected with the above strain of *T. brucei* 'O' and were killed with chloroform when the trypanosomes were numerous in the blood. Blood was withdrawn immediately by heart puncture into citrate saline solution. The trypanosomes were separated from the blood by differential centrifugation, and washed in modified Ringer-phosphate solution as described for *T. equiperdum* in a previous paper (Thurston, 1958*a*). The washed trypanosomes were suspended in modified Ringer-phosphate solution and the number of trypano-

* Present address: Department of Zoology, Makerere University College, Kampala, Uganda.

somes in the suspension was measured in an absorptiometer. The concentration was adjusted so that each flask contained $2{\cdot}4$–$4{\cdot}5 \times 10^7$ trypanosomes. A further sample of the suspension was diluted again and counted in a haemocytometer slide.

The oxygen uptake was measured with differential manometers using micro-flasks of 5 ml. capacity. The total volume of reaction mixture in each flask was 0·9 ml., and each flask also contained 0·1 ml. 15 % KOH in the centre well, absorbed on a roll of filter-paper. The flasks were equilibrated in air at 37° C. for 15 min., the taps were then closed and manometric readings commenced. During the period of equilibration the main flask contained the suspension of *T. brucei* in modified Ringer-phosphate solution with 0·02 M glucose; 0·1 ml. or 0·2 ml. serum or serum diluted with Ringer-phosphate solution was present in the side bulb of each flask. This serum was tipped into the reaction mixture immediately after the first manometric reading had been taken, and readings were continued at 15 min. intervals for up to $2\frac{1}{2}$ hr.

The trypanosomes were examined microscopically at the end of each experiment and the degree of motility was noted. In some instances, the suspension was also injected intraperitoneally into mice at the end of the manometric experiment, in order to test the infectivity of the trypanosomes. The blood of these mice was examined after 3–8 days.

The oxygen uptake of the trypanosomes was expressed as μl. $O_2/10^8$ trypanosomes/hr. The oxygen uptake of trypanosomes in the presence of immune serum was expressed as a percentage of the oxygen uptake with normal serum during the first 120 min. after serum was added to the reaction mixture. This gave the degree of inhibition of oxygen uptake caused by the immune serum.

RESULTS

The average oxygen uptake of *T. brucei* in the presence of 0·02 M glucose and 11 % normal rabbit serum was 135 μl./10^8 trypanosomes/hr. during the first 120 min. (average of nine experiments with extreme values 120 and 156 μl./10^8/hr.).

The results of experiments with the four immune sera are shown in Table 1. As the sera differed in their degree of activity, each set of experiments will be discussed separately.

Serum R 93. This serum was fairly active. At a concentration of 1/4·5 all the trypanosomes were lysed 120 min. after the serum was added; at 1/9 many of the trypanosomes were clumped together and activity had decreased, these trypanosomes were not infective to mice; at 1/20 there was no effect on motility, the trypanosomes were not clumped and they were infective to mice. The rate of decrease of oxygen uptake is shown in Fig. 1. This probably corresponds to the rate at which lysis is taking place and at the concentration of 1/4·5 occurs at the greatest rate between 15 and 75 min. after the addition of the immune serum.

Serum N 93. This serum was slightly active. At a concentration of 1/4·5 many of the trypanosomes were clumped and some were lysed, although the oxygen uptake during the first 2 hr. was not greatly reduced; they were not infective to mice. At concentrations of 1/9 and 1/20, only a few trypanosomes were clumped and the suspension was infective to mice.

Table 1. *The effect of different concentrations of four immune sera on the oxygen uptake, motility and infectivity of* Trypanosoma brucei *in vitro*

(Oxygen uptake in the presence of immune serum is shown as a percentage of the oyxgen uptake with normal serum ($\%O_2$). Infectivity (Inf.) is shown by I = mice infected; NI = mice not infected. Motility (Mot.) is expressed by the following symbols: −, all trypanosomes immotile; (+), occasional trypanosomes moving; +, decreased motility; ++, normal or very slightly decreased motility.)

		Concentration of sera in reaction mixture											
		1/4·5			1/9			1/20			1/100		
Immune serum	Expt.	$\%O_2$	Mot.	Inf.	$\%O_2$	Mot.	Inf.	$\%O_2$	Mot.	Inf.	$\%O_2$	Mot.	Inf.
R 93	1	.	.	.	71	+	NI	100	++	I	.	.	.
	2	37	−	.	75	+	.	.	.	.	.	.	.
	3	41	−	.	.	.	.	.	.	.	.	.	.
N 93	1	80	+	NI	90	++	I	90	++	I	.	.	.
M 5	1	.	.	.	85	++	.	.	.	.	.	.	.
	2	85	+	NI	86	+	NI	82	++	NI	.	.	.
	3	64	(+)	NI	90	++	NI	.	.	.	.	.	.
	4	50	(+)	.	.	.	.	.	.	.	.	.	.
OR 17	1	74	+	NI	34	(+)	NI	.	.	.	.	.	.
	2	.	.	.	.	.	.	.	.	.	100	++	I
	3	89	+	.	41	(+)	.	45	(+)	.	.	.	.
	4	83	+	.	41	+	.	28	(+)	.	.	.	.

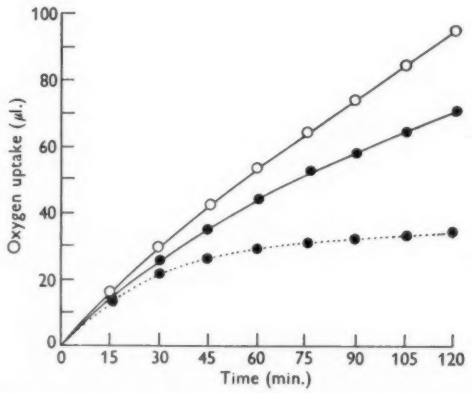


Fig. 1. Oxygen uptake of suspensions of *T. brucei* in modified Ringer-phosphate solution with 0·02 M glucose and normal rabbit serum or immune rabbit serum R 93. The sera were added at time 0 min. Each flask contained $3{\cdot}5 \times 10^7$ trypanosomes. ○—○, normal serum, concentration 1/9; ●...●, immune serum, concentration 1/4·5; ●—●, immune serum, concentration 1/9.

Serum M 5. In Expts. 1 and 2 these results were comparable with those described for serum N 93, except that trypanosomes in concentrations 1/9 and 1/20 in Expt. 2 were not infective to mice. A greater degree of activity was shown in Expts. 3 and 4. This was probably because there were fewer trypanosomes in

these experiments, only 2·5 and $3{\cdot}0 \times 10^7$ per flask, compared with $4{\cdot}5 \times 10^7$ per flask in Expt. 2.

Serum OR 17. This serum was the most active of the four immune sera. The maximum activity was found in concentrations 1/9 and 1/20, where most of the trypanosomes were lysed after 120 min. and the suspension was not infective to mice. There was little activity at a concentration of 1/100 and the trypanosomes were still infective to mice. At the highest concentration, 1/4·5, the serum had little effect on the oxygen uptake of the trypanosomes during the first hour, as is shown in Fig. 2. The oxygen uptake then decreased and many of the trypanosomes were lysed at 120 min. The suspension was then not infective to mice.

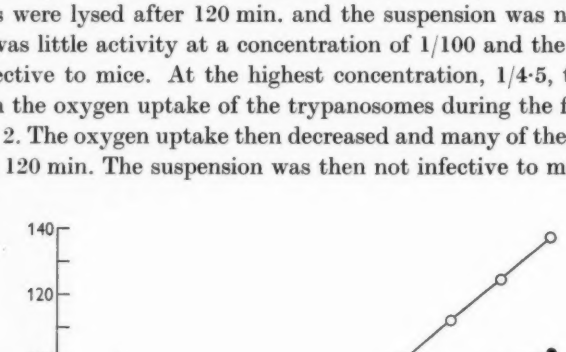


Fig. 2. Oxygen uptake of suspensions of *T. brucei* in modified Ringer-phosphate solution with 0·02 M glucose and normal rabbit serum or immune rabbit serum OR 17. The sera were added at time 0 min. Each flask contained $4{\cdot}0 \times 10^7$ trypanosomes. ○—○, normal serum, concentration 1/9; ●...●, immune serum, concentration 1/4·5; ●—●, immune serum, concentration 1/9; ●---●, immune serum, concentration 1/20.

DISCUSSION

During the present experiments it was found that the oxygen consumption of *T. brucei* was reduced only by those concentrations of immune serum that caused the trypanosomes to become non-motile and then to be lysed. The lower concentrations of serum that inactivated the trypanosomes (that is, made them non-infective to mice) without affecting their motility had little effect on the respiration of the trypanosomes.

Some concentrations of immune sera caused a proportion of the trypanosomes to be lysed or clumped together while other organisms appeared to be unaffected. In these circumstances, a reduction of oxygen uptake to 80–90% of that of trypanosomes in the presence of normal serum probably indicated the proportion

of affected trypanosomes and not a general reduction in the rate of respiration of the whole suspension.

There were great differences in activity between twofold dilutions of the sera. There were also considerable differences in oxygen uptake between different numbers of trypanosomes in the same concentration of immune serum. Similarly, Soltys was able to demonstrate much greater activity in these sera because he worked with trypanosomes at a concentration of only 50–100 organisms per ml., whereas in the present experiments the concentration was $3–5 \times 10^7$/ml. and the trypanosomes were only affected by the most active antisera. That is, the demonstration of neutralizing antibodies is a more sensitive method of determining the presence of immune bodies in serum than is the determination of the inhibition of oxygen uptake.

SUMMARY

1. Blood-stream forms of *Trypanosoma brucei* 'O' strain from infected rats were separated from the blood by differential centrifugation. After washing, the trypanosomes were suspended in modified Ringer-phosphate solution with 0·2 M glucose, and normal or immune rabbit serum was added. The oxygen uptake of such trypanosome suspensions was measured at 37° C. for 2 hr. with differential manometers using microflasks of 5 ml. capacity.

2. Immune sera that affected the motility of the trypanosomes also caused a decrease in oxygen uptake.

3. Some trypanosomes appeared to be unaffected by the less active concentrations of immune sera, while other trypanosomes in the same suspension were clumped together or lysed.

4. The most active serum inhibited oxygen uptake less at the highest concentration tested (1/4·5) than at lower concentrations (1/9 and 1/20).

5. This technique was less sensitive than Soltys's method (1957) for demonstrating neutralizing antibodies in the same sera.

I should like to thank Dr M. A. Soltys for suggesting this line of work and for providing the immune sera and the strain of *T. brucei*.

This work was carried out with the aid of a grant from the Agricultural Research Council.

REFERENCES

Desowitz, R. S. (1956). Effect of antibody on the respiratory rate of *Trypanosoma vivax*. *Nature, Lond.*, **177**, 132.

Soltys, M. A. (1957). Immunity in trypanosomiasis. I. Neutralization reaction. *Parasitology*, **47**, 375.

Thurston, J. P. (1958*a*). The respiration of *Trypanosoma lewisi* and *Trypanosoma equiperdum* with especial reference to oxygen uptake in the presence of amino-acids. *Parasitology*, **48**, 149.

Thurston, J. P. (1958*b*). The effect of some metabolic inhibitors on the oxygen uptake of *Trypanosoma lewisi* and *Trypanosoma equiperdum*. *Parasitology*, **48**, 165.

A COMPARISON OF THE STRUCTURE OF THE SCOLEX OF *BOTHRIOCEPHALUS SCORPII* (MÜLLER 1776) AND *CLESTOBOTHRIUM CRASSICEPS* (RUD. 1819) AND THE MODE OF ATTACHMENT OF THE SCOLEX TO THE INTESTINE OF THE HOST

By GWENDOLEN REES, D.Sc.

Department of Zoology, University College of Wales, Aberystwyth

(With Plates XVIII and XIX and 31 Figures in the Text)

Bothriocephalus (Rud. 1808) Lühe 1899 and *Clestobothrium* Lühe 1899 are two genera placed, as a general rule, in the family Ptychobothriidae Lühe 1902 of the order Pseudophyllidea. Cooper (1918), Joyeux & Baer (1936), Hyman (1951), and many others, accept this view, but Wardle & McLeod (1952) have placed them in separate families, the Bothriocephalidae Blanchard 1849 (emend. Wardle & McLeod, 1952) and the Ptychobothriidae respectively, mainly because the eggs are operculated in the former but not in the latter.

The present specimens of *Bothriocephalus scorpii* (Müller 1776) were obtained from the pyloric caeca and intestine of a single *Scophthalmus maximus* (L.), the turbot, caught in the trawl, on 6 July 1954, in the locality 54° 6′ N. and 11° 15′ W. at a depth of 143 fathoms. Those of *Clestobothrium crassiceps* (Rud. 1819) were found in the intestine of four specimens of *Merluccius merluccius* (L.), the hake, caught on 10 July 1954, in the locality 55° 55′ N. and 8° 25′ W. at a depth of 66 fathoms. For this material I am most grateful to Dr H. H. Williams of this department who collected it during an excursion on a commercial trawler, in July 1954, to the fishing grounds off the west coast of the British Isles. In addition, some specimens of *C. crassiceps* have been used which were deposited in this department by Dr J. Llewellyn in 1938 and collected by him from nearly the same locality, namely, 52° 55′ N. to 53° 30′ N. and 14° W. at a depth of from 160 to 200 fathoms.

The type host of *B. scorpii* is *Cottus scorpius* L., from which it was first recorded by Müller in Denmark. Cooper (1918) has indicated, in a table, that it had up to that date been reported from about fifty different species of fishes covering a wide area including all the coasts of Europe, the Atlantic coasts of Canada and the United States and the Arctic Ocean. Wardle (1933) recorded it from three more hosts and for the first time from Pacific waters, and Dollfus (1956), more recently, from the herring at Woods Hole (Massachusetts). It has been found in *Scophthalmus maximus* (L.) from many parts of Europe and has been reported from this host off the British Isles by Nicoll (1907) from the North Sea, by Bellingham (1844) from Ireland and off Penkilan Head in Wales by Hilmy (1929). Records of it from other hosts off the coasts of the British Isles are by Baylis (1939) at Plymouth, Nicoll (1907) from the North Sea, and Bellingham (1844) and Diesing (1850) from

Ireland. Wardle (1933) made the interesting statement that: 'The wide distribution and wide range of hosts of this cestode would suggest the existence of a *scorpii* group of bothriocephalid species, externally much alike, differing probably in minute internal details and in physiological adaptations to particular hosts.'

C. crassiceps is the type and only species of the genus. The type host is *Merluccius merluccius* (L.), it being first recorded by Rudolphi in 1819 from *Gadus merluccius* L. (=*M. merluccius*) at Naples. It has not been recorded as frequently as *B. scorpii*, but has a wide range of distribution in five species of host fishes off the coasts of Europe and America. It has been reported off the British Isles by Thompson (1844) in Ireland, Johnstone (1909) off the Calf of Man, Cooper (1918) at St Andrews, and Rees & Llewellyn (1941) from the Porcupine Bank. It was later found by Williams in 1954 from nearly the same area as the last named.

Cooper (1918) has given a description of both species and has included, in each case, a list of synonyms in addition to tables indicating the localities from which they must have been reported with the collectors' names. As is often the case, however, little attention has been paid to the detailed structure of the scolex. Niemiec (1888) and Lönnberg (1891) have made contributions in the case of *B. scorpii*, and Cooper (1918) appears to be the only author who has dealt, briefly, with the scolex of *C. crassiceps*. The musculature and nervous systems of this organ will, therefore, be described in each case, the relationship of the arrangement of the nerves to that of the muscles will be examined, and as the two species are closely related they will be compared. The mode of attachment of the scolex to the intestinal mucosa of the host will be described, more particularly, for *B. scorpii*.

All specimens were fixed, at the time of collecting, in 5% formaldehyde. Whole mounts of the scolices have been stained with Ehrlich's haematoxylin, Celestin blue or aceto-carmine. Series of transverse, horizontal and sagittal sections have been cut at 8 μ in thickness and stained with Heidenhain's Azan, Hansen's Trioxyhaematin or Ehrlich's haematoxylin and Orange G. Portions of the intestinal wall of *Scophthalmus maximus* with numerous specimens of *B. scorpii* still attached were cut out and fixed in 5% formaldehyde at the time of collecting. Some of these have been sectioned with the worms still *in situ* so that their mode of attachment and any effect they might have on the mucosa of the host could be examined.

EXTERNAL FEATURES OF THE SCOLEX OF *BOTHRIOCEPHALUS SCORPII* AND *CLESTOBOTHRIUM CRASSICEPS*

The scolex of *B. scorpii*, as noted by Cooper (1918), Hilmy (1929) and others, varies greatly in shape in preserved material. As a general rule, it is elongated with the greatest breadth near the middle region (Text-figs. 8, 9). The anterior end is usually, but not invariably, broader than the posterior. The measurements of the present specimens fall within the ranges given by Cooper (1918) and Hilmy (1929), namely, length 1·0–3·5 mm. and breadth 0·3–1·09 mm. The scolex terminates in a square disk indented on the bothrial edges, the two indentations being connected, usually, across the apex by a groove. A slight constriction exists behind the disk. Laterally a groove runs the length of the scolex (Text-fig. 9),

dividing it into a dorsal and a ventral bothrial area. On either side of the groove the lateral surface is convex as it curves dorsally and ventrally towards the free margins of the bothria (Text-figs. 2–7). The latter are long, narrow and deep in the expanded worm and short, wide and shallow in the contracted one. They are deepest a short distance behind the terminal disk (Text-figs. 3, 4), becoming shallower and narrower posteriorly (Text-fig. 2).

The type of scolex found in *C. crassiceps* (Text-fig. 11) could be derived from that of *B. scorpii* by a shortening of the scolex and a rolling together and fusion of the margins of each bothrium along the greater part of its length, leaving only a small opening near the apex which leads into a deep hemispherical cavity. Instead of being elongated the scolex of *C. crassiceps* is almost spherical or pear-shaped, narrowing slightly, anteriorly, in both surface and lateral views. There is some variation in the shape relative to the degree of expansion or contraction, but this is not as extreme as in *B. scorpii*. The measurements fall within the range given by Cooper (1918), namely, length 0·64–1·08 mm., breadth 0·52–0·90 mm., and depth 0·68–1·21 mm. There is a lateral longitudinal groove on either side as in *B. scorpii* which separates the dorsal and ventral hemispherical bothria (Text-figs. 11, 15–20). The convex lateral wall on either side of each groove passes insensibly into the wall of the bothrium. The bothria are large and prominent, oval in surface view and more or less conical in lateral view (Text-fig. 26), as the dorso-ventral diameter of the bothrium is greater posteriorly than anteriorly. This accounts for the pear-like shape of the scolex as a whole. The opening of each bothrium occupies, usually, about a third of the length of the scolex from the anterior end, but the size and shape of the opening varies somewhat in preserved specimens according to the degree of contraction of the muscles at the time of fixation. It is surrounded by a sphincter muscle (Text-figs. 23, 24, 27). The bothria are deep, more or less hemispherical in shape with fairly thick walls and may project slightly, posteriorly, beyond the true limit of the scolex. The two openings of the bothria are connected, across the apex of the scolex, by a saddle-shaped groove (Text-figs. 25, 27).

There is no neck in either species, segmentation beginning immediately behind the scolex.

The cuticle in both consists, over the scolex and storbila, of two layers as observed by Hilmy (1929), Markowski (1935) and others. The outer is composed of closely set, backwardly projecting structures which have been variously named 'pseudocilia', 'cirri' or 'hairs', and which stain more readily than does the inner homogeneous layer. The cuticle is thinner on the scolex than elsewhere and measures, on the scolex and strobila respectively, 4–6 μ in *B. scorpii* and 2–5 μ in *C. crassiceps*.

MUSCULATURE OF SCOLEX

Bothriocephalus scorpii. Lönnberg (1891) gave a brief account of the musculature in this species. For a complete account of the musculature of the scolex, which is derived in the main from the muscles of the strobila, the arrangement immediately behind the scolex will be examined and the muscles followed forwards. In the Pseudophyllidea the bothria are not sharply demarcated from the under-

lying parenchyma and, as in *B. scorpii*, they are in the form of open grooves, the muscles of the strobila continue directly into them and form an integral part of their structure with little modification.

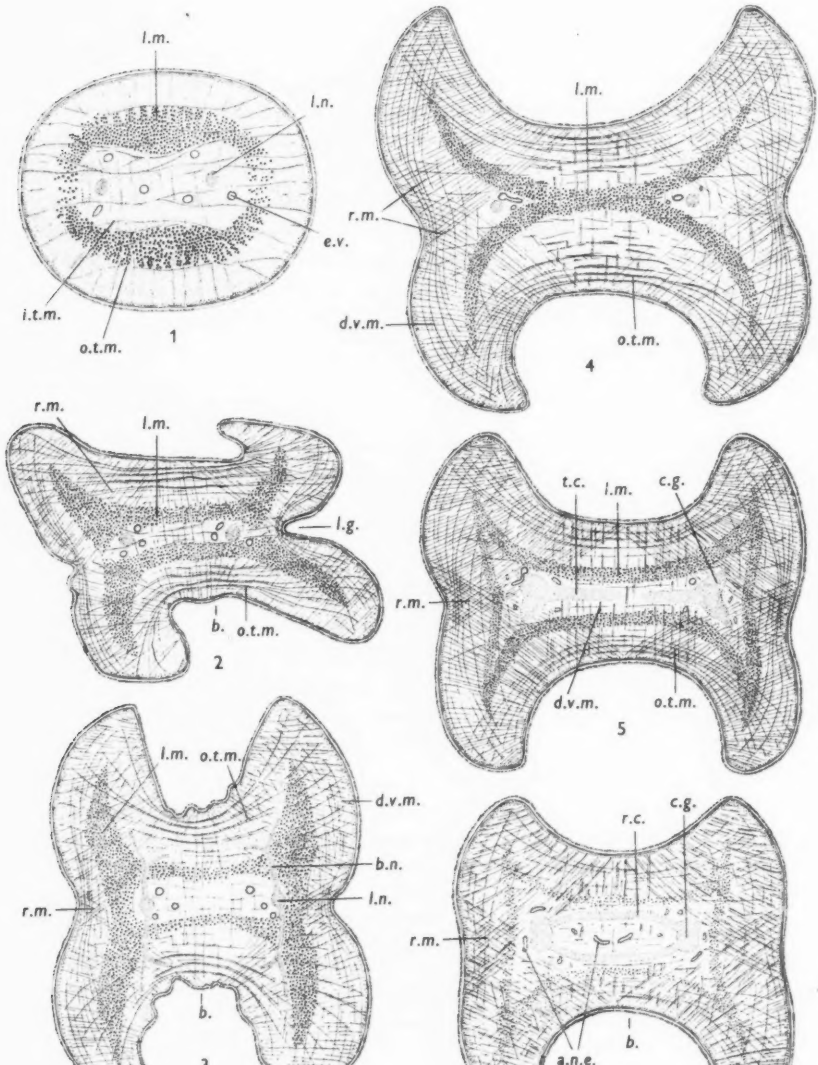
(*a*) *Superficial musculature.* The superficial musculature immediately behind the scolex consists of a single outer layer of circular muscles followed by an inner layer of longitudinal muscles several fibres deep (Text-fig. 1). These continue into the scolex. In the cavity of each bothrium the superficial longitudinal muscles consist of a single row of fibres while laterally there are several rows, the transition taking place fairly suddenly at the margins of the bothria (Text-figs. 2–7).

(*b*) *Deep musculature.* The deep musculature, behind the scolex, consists of longitudinal muscles, inner and outer transverse muscles, dorso-ventral and transverse muscles in the medulla and radial muscles in the cortex (Text-fig. 1.). The longitudinal muscles are arranged in bundles forming a broad band dorsally and ventrally, but narrower laterally. The outer and inner transverse muscles lie to the outer and inner sides of the longitudinal muscles, but a few fibres are enclosed within the longitudinal muscle area. They are feebly developed, spread out laterally in the cortex and are inserted into the basement membrane. The longitudinal and transverse muscles anastomose with neighbouring fibres.

Owing to the simplicity of the structure of the scolex the muscle layers do not become profoundly modified on entering this organ. As already mentioned, the bothria are deeper anteriorly and so the greatest change would be expected at the anterior end.

(1) *Longitudinal muscles.* A transverse section through the posterior end of the scolex shows the bothria as broad shallow grooves, the lateral longitudinal groove is also apparent on either side (Text-fig. 2). The longitudinal muscles enter the scolex as a ring of bundles. With the appearance of the bothria the ring expands and becomes indented dorsally and ventrally, taking on a shape parallel to that of the cavity of the bothrium. The muscles are still continuous laterally, but it is now difficult to recognize bundles as the fibres form a fairly uniform band. A layer, or 'horn', of longitudinal fibres extends into the margin of the bothrium dorsally and ventrally on either side. The longitudinal muscles surround a more or less rectangular area, in the centre, containing the lateral nerves and excretory vessels. Continuing forwards the bothria become narrower and deeper with more prominent margins (Text-fig. 3). The longitudinal muscle layer consequently shows a more pronounced curvature dorsally and ventrally and is still continuous laterally to the outer side of the lateral nerves. This arrangement persists along the length of the scolex to just behind the nerve commissures where, for a short distance, the dorsal and ventral muscle layers become continuous, in the centre of the scolex, separating the lateral nerves and excretory vessels of the right and left sides (Text-figs, 4. 10). At first the longitudinal muscles are complete laterally but soon separate into a distinct dorsal and ventral band joined in the centre in the form of a × (Text-fig. 4). In the region of the nerve commissures (Text-figs. 5, 6) the dorsal and ventral layers must separate. Some of the fibres extend laterally so that there is once again a continuous but narrow layer of muscle fibres to the outer side of the cerebral ganglia. In front of the nerve commissures the longitudinal muscle

fibres become more diffuse and more delicate. They spread out and continue into the apical disk where some of them are inserted into the basement membrane, while others cross the apex dorso-ventrally, or laterally, to become continuous with their fellows of the opposite side. Contraction of these muscles will shorten the scolex.



Text-figs. 1–6. *Bothriocephalus scorpii* (Müller).

For legends see opposite page.

(2) *Outer transverse muscles.* On entering the scolex the outer transverse muscles are feebly developed. They extend into the cortex and are inserted into the basement membrane laterally. With the appearance of the bothria these muscles become much more powerful (Text-fig. 2). They are arranged in bundles of two to a few fibres, the outermost following the concavity of the bothrium and the inner ones running more or less straight across the scolex. They diverge, therefore, laterally, passing between the longitudinal muscle fibres of the 'horns' and continue forwards throughout the length of the scolex following the concavity of each bothrium (Text-figs. 3–7). They are well developed up to the level of the nerve commissures, but in front become more delicate and in the apical disk form, with the inner transverse muscles, a uniform layer of fine fibres.

(3) *Inner transverse muscles and transverse muscles of the medulla.* These remain fairly delicate throughout the length of the scolex (Text-figs. 2–7). They cross from one side of the scolex to the other, passing close to the lateral nerves and excretory vessels. A few are present in the region where the dorsal and ventral longitudinal muscles meet in the centre, weaving their way across between the longitudinal fibres (Text-fig. 4). At the anterior extremity they contribute to the general network.

(4) *Dorso-ventral muscles.* Dorso-ventral muscles are present in the medulla at the base of and immediately behind the scolex (Text-fig. 1). They are continuous with the radial muscles dorsally and ventrally, and are confined mainly to the central zone between the lateral nerves and excretory vessels. A few, however, are present outside the lateral nerves (Text-fig. 2). Proceeding forwards the dorso-ventral muscles become better defined, forming a uniform band across the centre where they take a straight course from the dorsal to the ventral side. They also extend further laterally where they follow the curve of the lateral wall of the scolex and are inserted into the interno-lateral wall of the margin of each bothrium (Text-figs. 3–7). They continue forwards in this manner, being best developed in the muscular region behind and in the neighbourhood of the nerve commissures (Text-figs. 4–6), especially in the central zone. In front of the nerve commissures they become more delicate and cross the transverse fibres.

(5) *Radial muscles.* The radial muscles at the base of the scolex are extremely

---

Text-fig. 1. Transverse section immediately behind the scolex showing the arrangement of the muscles, nerves and excretory vessels.

Text-fig. 2. Transverse section through the posterior end of the scolex showing the shallow cavity of each bothrium and the muscles of this region.

Text-fig. 3. Transverse section through the anterior third of the scolex showing the deepening of the cavity of each bothrium, the origin of the bothrial nerves and the greater development of the musculature.

Text-fig. 4. Transverse section through the scolex a short distance behind the nerve commissures, showing the joining of the dorsal and ventral longitudinal muscles in the centre. Other muscles well developed.

Text-fig. 5. Transverse section through the scolex showing the bilobed cerebral ganglia, the transverse commissure and the musculature.

Text-fig. 6. Transverse section through the scolex showing the ring commissure and the musculature.

delicate fibres radiating out through the cortex, some being continuous with the dorso-ventral and others with the transverse fibres. Passing into the scolex and with the appearance of the bothria and lateral longitudinal grooves, the position of some of the radial muscles becomes altered. They now extend from the cavity of the bothrium across to the lateral wall of the scolex, forming, as it were, four sets of diagonal muscles running in the free margins of the bothria (Text-fig. 2). At first, these four groups of muscles are distinct and do not encroach upon one another, but passing forwards those of the dorsal and ventral sides cross one another on the outer side of the lateral nerves and extend, slightly, into the apposite halves of the scolex on either side (Text-figs. 3–7). Near the nerve centre they also encroach on the central zone, dorsally and ventrally, meeting in the middle line (Text-figs. 5–7) and crossing the dorso-ventral muscles. They become more delicate again, as do the other muscle fibres, at the anterior extremity and are seen to cross the 'corners' of the apical disk.

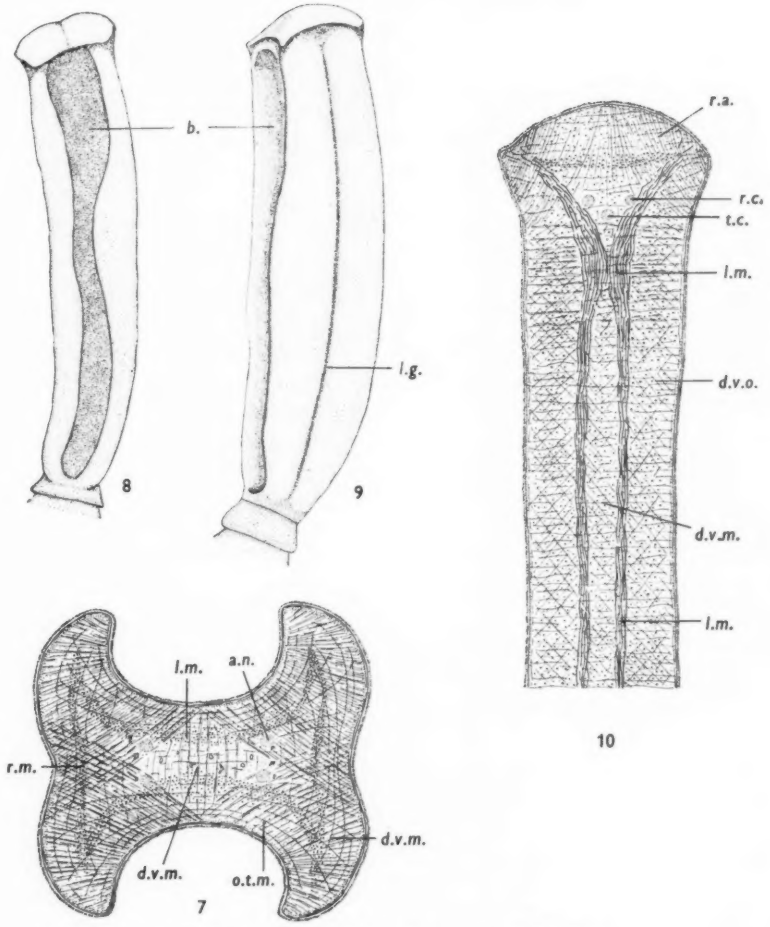
The concerted action of the outer and inner transverse, the dorso-ventral and the radial muscles, would bring about changes in the shape of the bothria, some deepening the cavity and others opening and closing the grooves. The arrangement seems to be a most efficient one for the purpose. The part of the scolex where all the muscles are most strongly developed is the anterior region in the neighbourhood of the nerve commissures (Text-figs. 4, 5, 10). This, probably, is the region which has the most powerful adhesive action.

Two other sets of muscles are present in the scolex which do not appear as such in the strobila and which may be modifications of the radial muscles. They are (1) the radiating apical muscles, and (2) the oblique or diagonal muscles. The former are present in the apical disk where they radiate from the centre backwards and outwards to be inserted into the body wall in the region of the brain (Text-fig. 10). The oblique or diagonal muscles are in two series: (*a*) a double dorso-ventral series running from the dorsal to ventral sides of the scolex crossing one another and best developed in the central zone (Text-fig. 10); and (*b*) a double lateral series running from side to side mainly at the base of the margins of the bothria.

Clestobothrium crassiceps. Cooper (1918) has given an outline of the arrangement of some of the muscles in the scolex. Owing to the fusion of the margins of each bothrium to enclose a hemispherical cavity and to the development of a sphincter muscle around the aperture, the arrangement of the muscles is a little more complicated in this species than in the former. The bothria are again not sharply demarcated from the underlying parenchyma and the muscles of the strobila run forwards into the scolex, entering into the composition of the musculature of the bothria. The differences which exist between this and *B. scorpii* can easily be related to the form of the scolex and the function of its various parts. As before, the arrangement of the muscles immediately behind the scolex will be examined and they will be followed forwards into that organ.

(*a*) *Superficial musculature.* The superficial musculature behind the scolex consists of a single layer of circular fibres followed by a single layer of longitudinal fibres (Text-fig. 12). These continue into the scolex and remain unchanged over the whole of its surface (Text-figs. 13–20).

(*b*) *Deep musculature*. The deep musculature consists of the same elements as in *B. scorpii*, namely, longitudinal muscles, inner and outer transverse muscles, dorso-ventral and transverse muscles in the medulla and radial muscles in the cortex (Text-fig. 12). The longitudinal muscles are again arranged in a band, broad dorsally and ventrally and narrower laterally, encircling the medulla. The delicate inner and outer transverse muscles lie to the inner and outer sides of these respectively, and are inserted, laterally, into the basement membrane. The transverse



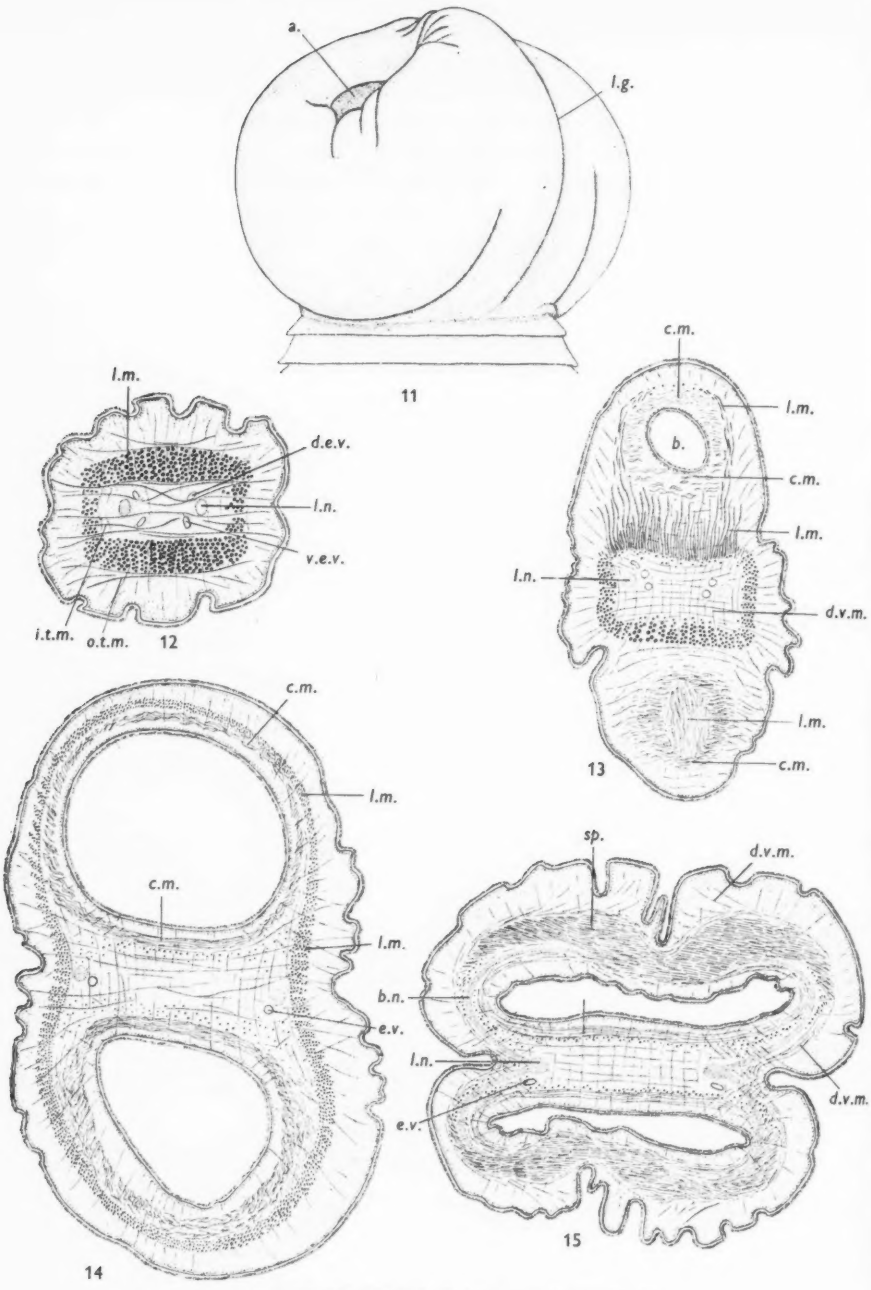
Text-figs. 7–10. *Bothriocephalus scorpii* (Müller).

Text-fig. 7. Transverse section through the scolex, in front of the nerve commissures, showing the four anterior nerves and the musculature.

Text-fig. 8. Scolex, dorsal or ventral view.

Text-fig. 9. Scolex, lateral view.

Text-fig. 10. Sagittal section through the scolex showing the joining of the dorsal and ventral muscles. The radiating apical muscles and dorso-ventral oblique muscles are also shown.



Text-figs. 11–15. *Clestobothrium crassiceps* (Rud.).

For legends see opposite page.

muscles of the medulla are continuous with the inner transverse muscles and pass across close to the nerve cords and excretory vessels. Anastomoses are present between the fibres comprising the different groups with the exception of the radial fibres.

As the scolex is less simple in form than in *B. scorpii*, it would be expected that the musculature, also, would be less simple. The main differences between the two are the shortening of the scolex in *C. crassiceps* and the fusion of the margins of the bothria along almost the whole of their lengths, leaving a small aperture anteriorly. There is, therefore, an outer wall to the bothrium which is provided with muscles. Even so, as in other Pseudophyllidea, the musculature is not sharply marked off from the parenchyma and is not as concentrated as in the suckers of the Cyclophyllidea. Some kind of sphincter muscle is therefore necessary around the aperture of the bothrium if it is to function adequately.

(1) *Longitudinal muscles.* As the longitudinal muscles enter the scolex the ring takes on a more rectangular shape as seen in transverse section (Text-fig. 13), being again slightly broader dorsally and ventrally than laterally. The fibres are more uniformly arranged instead of being in bundles. At the bases of the bothria dorsally and ventrally the longitudinal muscles do not all continue straight forwards as in *B. scorpii* but, owing to the presence of an outer wall, they divide into two layers. The outer layer, which is the broader, curves around the posterior margin of the cavity of the bothrium (Text-figs. 13, 26), and then extends forwards in the outer wall (Text-figs. 14, 15, 26), most of the fibres passing eventually on either side of the aperture of the bothrium. These muscles are usually cut obliquely in a transverse section of the scolex. The inner layer continues straight up the centre of the scolex as fibres lying near the inner wall of the bothrium (Text-figs. 13, 26). The longitudinal muscles are still present laterally and continue forwards for some little distance so that at the base of the scolex the bothria and central zone are surrounded by a continuous sheath of longitudinal muscles (Text-figs. 13, 14). This condition could be derived from that obtaining in *B. scorpii* by the fusion of the free margins of the bothria and the continuation of the longitudinal muscles across the outer wall so formed. Passing forwards, at about half-way along the length of the scolex, the lateral longitudinal muscles divide so that they no longer form a well-marked layer. Each bothrium is now surrounded by a separate layer of longitudinal fibres (Text-fig. 15). Such a separation is visible in *B. scorpii*, but only for a very short distance just behind the nerve commissures. A few isolated

d.v.m.

d.v.m.

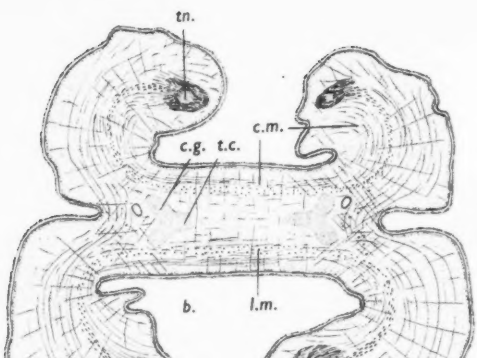
Text-fig. 11. Scolex, dorso-lateral or ventro-lateral view.

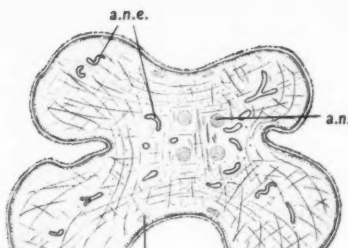
Text-fig. 12. Transverse section immediately behind the scolex showing the arrangement of the muscles, nerves and excretory vessels.

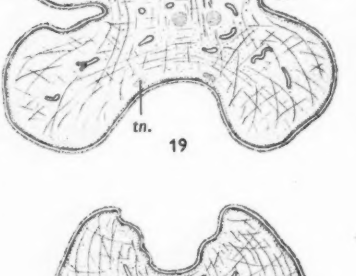
Text-fig. 13. Transverse section through the bases of the bothria showing (*a*) the outer longitudinal muscles curving into the outer wall of the bothrium, and (*b*) the outer transverse muscles separating around the bothrial cavity to form the circular muscles.

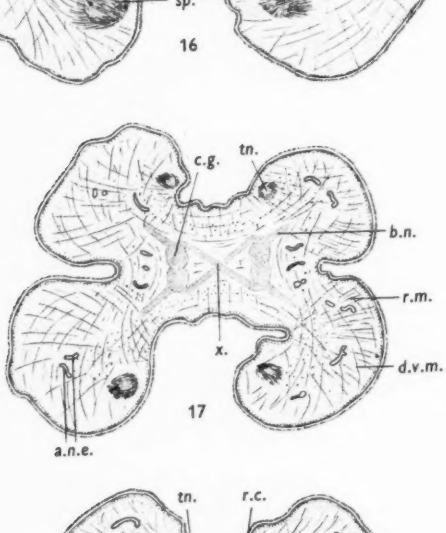
Text-fig. 14. Transverse section through the posterior half of the scolex showing the bothria and the arrangement of the muscles.

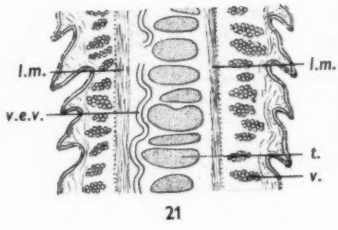
Text-fig. 15. Transverse section through the scolex just below the bothrial aperture showing part of the sphincter muscle and the origin of the bothrial nerves.

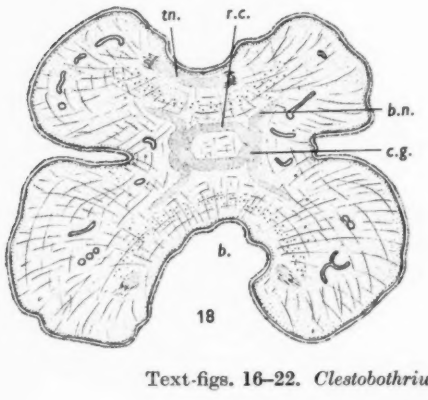


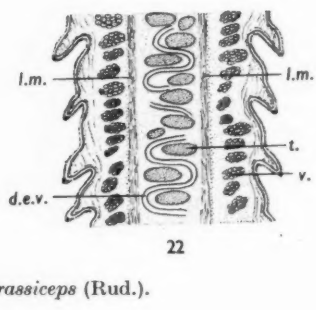












Text-figs. 16–22. *Clestobothrium crassiceps* (Rud.).

For legends see opposite page.

longitudinal fibres, however, continue forwards, laterally, to the outer side of the lateral nerves and excretory vessels (Text-figs. 15, 16). The longitudinal fibres in the central zone of the scolex are at first in a layer to the inner side of the circular muscles (Text-figs. 13, 14, 26), but anteriorly the layer loses its continuity and the fibres tend to become mixed with the transverse and circular fibres (Text-figs. 16–18). They continue forwards to the apex of the scolex where they spread out fanwise and are inserted into the basement membrane at the apex (Text-fig. 26). The few remaining lateral fibres behave in the same way. The course of some of the longitudinal muscles in the outer wall of the bothrium is bound up with the sphincter muscle which surrounds the aperture of the bothrium and which will be referred to later.

(2) *Outer transverse muscles.* Surrounding the cavity of the bothrium in the posterior half of the scolex is a layer of circular muscles derived, apparently, from the outer transverse muscles of the strobila (Text-figs. 13–15). These take a slightly oblique course, passing more anteriorly in the outer wall of the bothrium. A transverse section through the posterior wall of the bothrium shows a broad band of fibres running transversely. These divide on either side of the cavity (Text-fig. 13), forming, as one passes forwards, the circular muscle layer. In the central zone of the scolex they are mainly on the outer side of the longitudinal muscles, that is, next to the cavity of the bothrium (Text-figs. 14, 15). The same is true, actually, all around the bothrium, but due to the obliquity of these and to a certain extent, also, of the longitudinal muscles, the limit of the layers is somewhat difficult to determine. In the region of the bothrial aperture, which faces obliquely outwards, a gap in the circular muscles, on the outer side naturally appears, in transverse section (Text-figs. 16–19). Here some of them are inserted into the sphincter and others into the wall around the inner margin of the aperture (Text-fig. 16). This arrangement more closely resembles that of *B. scorpii*, the outer transverse muscles following the concavity of the bothrium which in transverse section now has the appearance of an open ground (Text-figs. 16–20). Passing towards the apex of the scolex the outer transverse muscles become delicate, spread out fanwise laterally and contribute to the general delicate network at the apex (Text-figs. 19, 20).

(3) *Inner transverse muscles and transverse muscles of the medulla.* These, together,

Text-fig. 16. Transverse section through the anterior part of the scolex showing the apertures of the bothria, portions of the sphincter and other muscles, the bilobed ganglia and origin of the transverse commissure.

Text-fig. 17. Transverse section through the scolex showing the cerebral ganglia, × -shaped commissure, origin of second pair of bothrial nerves, the muscles and excretory vessels.

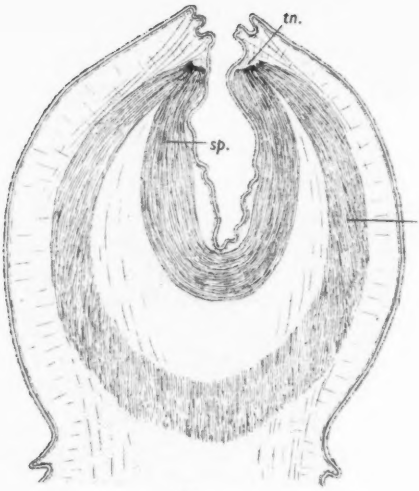
Text-fig. 18. Transverse section through the scolex showing the cerebral ganglia, ring commissure, origin of the first pair of bothrial nerves, the muscles and excretory vessels.

Text-fig. 19. Transverse section through the anterior region of the scolex showing the four anterior nerves.

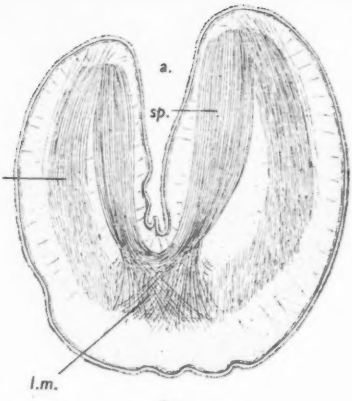
Text-fig. 20. Transverse section through the apex of the scolex showing the apical network of muscles and excretory vessels.

Text-fig. 21. Sagittal section through the strobila showing, mainly, the ventral excretory vessel.

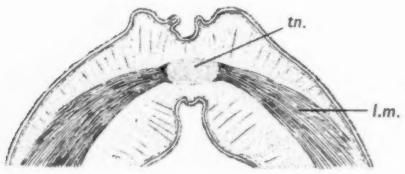
Text-fig. 22. Sagittal section through the strobila showing mainly the dorsal excretory vessel.



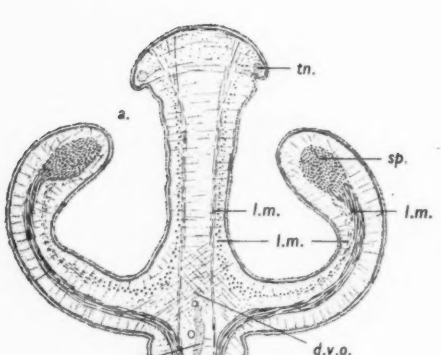
23



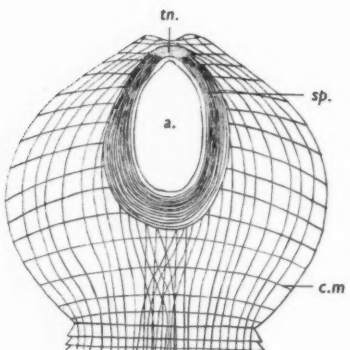
24



25



26



27

Text-figs. 23–27. *Clestobothrium crassiceps* (Rud).

For legends see opposite page.

extend across the medullary region from the dorsal to the ventral longitudinal muscles, passing out laterally to the margins of the body (Text-fig. 12). They continue forward throughout the scolex, showing little change and eventually contribute to the apical network (Text-figs. 13–20).

(4) *Dorso-ventral muscles.* These are present in the medulla behind the scolex (Text-fig. 12). In the scolex they can still be seen in the central zone passing from the inner wall of one bothrium across to the other (Text-figs. 14–16). They are also present in the neighbourhood of and outside the lateral nerves and excretory vessels as far as the lateral longitudinal muscles. At first, they are continuous dorsally and ventrally with the circular (outer transverse) muscles, an arrangement which is somewhat different from that in *B. scorpii*, where the dorso-ventral muscles extend evenly laterally into the margins of the bothria crossing the outer transverse muscles. In the present case, due to the closed nature of the bothrium, a more efficient arrangement is achieved by the continuity of the dorso-ventral and the circular muscles to form a powerful layer around the two bothria. Further forwards, however, where the longitudinal muscles separate laterally (Text-figs. 15–17), some of the dorso-ventral fibres extend outwards, a few passing around the bothrium to the outer side of the longitudinal muscles to be inserted into the wall on the outer side of the bothrial opening. Near the anterior end of the opening the dorso-ventral fibres are more uniformly distributed (Text-figs. 19, 20), as they are in *B. scorpii*, due presumably to a similarity in shape of the bothrium in this region. In front of the nerve commissures they tend to form a slightly more concentrated 'bundle' passing across the centre near the apex of the scolex (Text-fig. 20). Unlike *B. scorpii*, the fibres remain uniformly delicate throughout the length of the scolex, as it is not their function to deepen, by contraction, an open groove.

(5) *Radial muscles.* Delicate radial muscles are present in the cortex behind the scolex (Text-fig. 12). These continue into the wall of the bothrium. Proceeding forwards, they become better developed laterally (Text-figs. 14–18), those from the dorsal and ventral sides crossing one another in the neighbourhood of the lateral nerves and of the transverse commissure (Text-figs. 15, 16). They are well developed in the region around the bothrial opening and are recognizable as delicate fibres right to the apex of the scolex (Text-figs. 17–20).

All these major groups of muscles are present in both *B. scorpii* and *C. crassiceps*.

Text-fig. 23. Horizontal section through the outer wall of the bothrium showing the deeper part of the sphincter muscle and some of the longitudinal muscles.

Text-fig. 24. Horizontal section through the outer wall of the bothrium showing the more superficial part of the sphincter and the incorporation into it of some of the longitudinal muscles.

Text-fig. 25. Horizontal section through the anterior margin of the bothrium showing the tendon.

Text-fig. 26. Sagittal section through the scolex showing the division of the longitudinal muscles into two layers. Parts of the sphincter, tendon and dorso-ventral oblique muscles also shown.

Text-fig. 27. Diagram illustrating the incorporation of longitudinal and circular muscles into the sphincter muscle, surface view.

Their modifications have been somewhat different in the scolices of both species relative to the functions which they fulfil. It is, however, possible to derive the arrangement found in *C. crassiceps* from the more simple one of *B. scorpii*.

Of the two other sets of muscles which were present in the scolex of *B. scorpii*, one is also represented in *C. crassiceps*, namely, the oblique or diagonal muscles, and of these only the double-ventral series, which cross the medulla from the dorsal to the ventral sides and are best developed near the posterior limits of the bothria (Text-fig. 26).

(6) *Sphincter muscle*. The muscle not represented in *B. scorpii* and present in *C. crassiceps* is the sphincter muscle around the aperture of each bothrium (Text-figs. 15–18, 23–27). As the openings face antero-dorsally and antero-ventrally, respectively, the sphincter muscle is obliquely placed to the longitudinal axis of the scolex. It is not a completely isolated structure as some of the other muscles of the scolex are inserted into its outer margin. Towards the inner side the sphincter forms a broad band of concentric muscle fibres encircling the aperture (Text-fig. 23). Anteriorly the sphincter narrows slightly and is inserted into a small tendon extending across the anterior margin of the bothrial opening (Text-figs. 16–19, 23, 25–27). The other muscles incorporated into the sphincter are the longitudinal and circular muscles.

Some of the longitudinal muscles entering the scolex from the strobila, as already mentioned, curve into the outer wall of the bothrium. Mid dorsally and mid ventrally, that is immediately behind the bothrial opening, some of the longitudinal muscles pass into and form a component part of the more superficial region of the sphincter (Text-figs. 24, 27). Some of these pass straight into the sphincter muscle, while others first cross from one side to the other. Other longitudinal muscles passing up to the right and left of the middle line become inserted at different levels into the anterior half of the sphincter (Text-figs. 24, 27), while more laterally still a few are inserted directly into the tendon at the anterior margin of the bothrial aperture (Text-figs. 23, 25, 27); the remainder pass up to the apex of the scolex with the lateral longitudinal fibres (Text-fig. 27). The circular muscles in the neighbourhood of the sphincter behave in the same way. They pass obliquely forwards in the outer wall and are inserted into the sphincter muscle on either side along the whole of its length (Text-fig. 27). The simultaneous contraction of these longitudinal and circular muscles would enlarge the aperture, while contraction of some of them in various positions would alter the direction in which the aperture faces. Contraction of the sphincter itself would close the aperture.

The whole arrangement of the muscles of the scolex seems to be such that maximum efficiency can be achieved in an adhesive organ of this kind

THE MODE OF ATTACHMENT OF THE SCOLEX OF *BOTHRIOCEPHALUS SCORPII* AND *CLESTOBOTHRIUM CRASSICEPS* TO THE INTESTINE OF THE HOST

The shape of the bothria in *C. crassiceps* suggests that they may function in much the same way as the acetabula in the Cyclophyllidea, that is, by drawing in, by suction, a 'plug' of the host's mucosa into the cavity. Many specimens have been

found in which the cavity contains portions of the host's mucosa (Pl. XVIII, figs. 1, 2) which were torn away when the worm was removed. It seems doubtful, however, whether the musculature of the bothrium would be sufficiently powerful to pull up a 'plug' of the mucosa from a relatively flat surface as is the case in *Tetrabothrius affinis* from the whale (Rees, 1956). It would be more likely that in the intestine of *Merluccius merluccius* processes of some kind arise from the mucosa and portions of these could be fairly easily engulfed by the bothria, the muscles of the wall being, presumably, strong enough for such a purpose. The sphincter muscle around the aperture would then contract around the engulfed 'process', anchoring the scolex firmly in position. This is a supposition as, unfortunately, no portion of the intestine of *M. merluccius* was available for study and none of the specimens was fixed *in situ*.

In the case of *B. scorpii*, however, specimens were fixed still attached to the intestine of the host *Scophthalmus maximus*. The bothria of *B. scorpii* do not seem at first to be very efficient organs of attachment. The worms, however, were found to adhere very firmly to the intestine of the host and did not release their hold on fixation *in situ*. Pl. XVIII, fig. 3, shows a large number attached to a small portion of intestine. The infestation is so heavy that no part of the surface of the mucosa itself is visible. Portions of the intestine of *Scophthalmus maximus* taken from immediately behind the pyloric caeca were examined and sectioned with the worms in position in order to find out how this attachment was achieved. The form and structure of the intestinal mucosa are presumably closely bound up with the form of the scolex and consequently with the mode of attachment of the worm. One would expect, therefore, a certain type of adhesive organ to be associated with a certain type of mucosa. This, incidentally, might prove to be an important factor in host specificity in cestodes and also in the location of the worm within the gut of the host.

The inner surface of the intestine of *S. maximus*, immediately behind the pyloric caeca, is raised into a series of close parallel folds or ridges measuring up to 3 mm. in height (Pl. XVIII, fig. 4). These, the primary folds, appear to run in a longitudinal direction. Most of them are fairly straight, some slightly sinuous and occasionally neighbouring folds may join one another. They are permanent structures, not being flattened by distension of the intestine. The entire surface of the mucosa on both sides of the primary folds is again raised into secondary folds measuring up to 1 mm. in height. These are sinuous and run, mainly, obliquely down the sides, or along, the primary folds (Pl. XVIII, fig. 4., Pl. XIX, figs. 6, 7). As the primary folds are very close together, the secondary folds at the bases of those adjacent to one another may fuse to form tunnels of varying length (Pl. XIX, fig. 7).

The scolex of *B. scorpii* seems admirably suited for adhering to such a surface. The scolex is deeply embedded between the primary folds (Pl. XVIII, fig. 5), lying parallel with them (Pl. XIX, figs 6, 7). The bothrium then fits along and over the edge of one of the secondary folds, the margins contracting to grip it like an elongated pincer (Pl. XIX, figs 6, 7). As the primary folds are close together it is possible for the dorsal bothrium to grip the secondary fold of one, and the ventral the secondary fold of another adjacent to it. The most muscular part of the bothrium is the

anterior end a short distance behind the nerve commissures, and it is probably this region which is most firmly attached. Occasionally, two adjacent scolices may be attached to one another by one bothrium and to the mucosa by the other. One such instance is shown in Pl. XIX, fig. 7, the bothrium of one closing over the margin of the bothrium of the other. Scolices were also found to penetrate into the tunnels formed by the fusion of secondary folds and to be attached within these tunnels. It seems, therefore, that in this particular case there is a close correlation between the structure of the scolex and the form of the host's mucosa, the scolex being perfectly adapted to the surface to which it must adhere. If this particular structure in confined to this region of the intestine, it might account for the presence of the worms here and their absence from the more posterior part. What form the mucosa takes in the other hosts of this cestode is not known, but it would be interesting if it was similar in all cases.

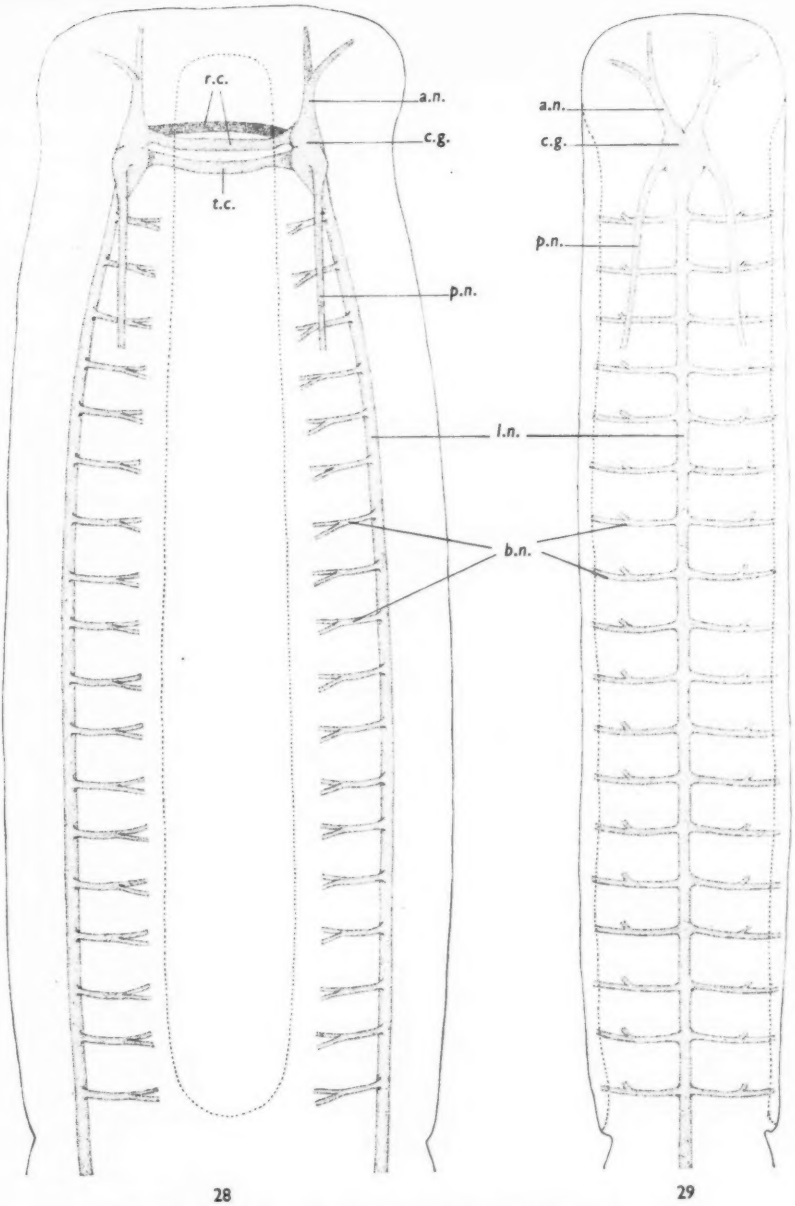
While considering the mode of attachment, the effect of the worms on the intestine might be examined. The epithelium covering the secondary folds consists of a single layer of well-defined cells attached to a basement membrane. The cells are of two kinds, namely, columnar ciliated cells and goblet mucous cells. The latter seem very abundant (Pl. XIX, fig. 8). The core of both primary and secondary folds is formed mainly of fibres. When a fold of mucosa becomes enclosed within the cavity of a bothrium, the depth of the epithelial layer covering it decreases to about half or less (Pl. XIX, fig. 8). The outlines of the cells are lost, but the mucus in the goblet cells is visible in places. Otherwise, there is little change except for a slight increase in the fibrous tissue in the core of the secondary fold. The alterations in the mucosa are not nearly as profound as, for example, in the intestine of the whale to which *Tetrabothrius affinis* is attached (Rees, 1956). This is what might be expected in the less powerful type of adhesive organ found in *B. scorpii* where the musculature is not highly developed. There are no hooks, no apical organ, and apparently no glands.

NERVOUS SYSTEM OF SCOLEX

The nervous system is intimately connected with the musculature and there should obviously be a correlation between the type of adhesive apparatus and the nerves which supply its constituent parts. The scolices of the two species now being considered differ in some details of structure, but have many points in common. One would expect, therefore, a fundamental similarity in the nervous system in the two with minor differences in the distribution of the nerves.

Bothriocephalus scorpii. The nervous system consists of a nerve centre at the apex of the scolex, lateral nerves passing backwards through the scolex and into the strobila, bothrial nerves to the bothria, anterior nerves to the anterior extremity and posterior nerves to the most muscular region of the scolex (Text-figs. 28, 29).

Niemiec (1888) and Lönnberg (1891) have given brief accounts of the nervous system which do not agree in all points with that found in the present instance. Near the apex of the scolex and a short distance behind the apical disk, there is on either side a bilobed cerebral ganglion (Text-figs. 5, 28, 29). These are connected



Text-figs. 28, 29. *Bothriocephalus scorpii* (Müller).

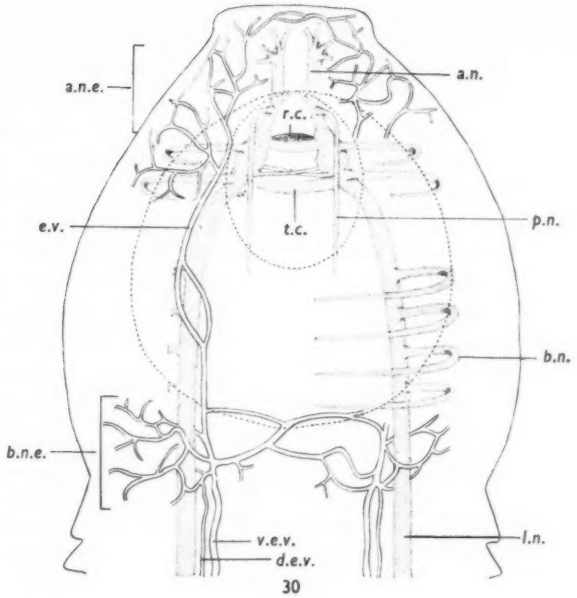
Text-fig. 28. Nervous system of scolex, dorsal or ventral view.
Text-fig. 29 Nervous system of scolex, lateral view.

with one another by a median transverse commissure and a short distance in front by a ring commissure which is somewhat hexagonal in shape (Text-figs. 5, 6, 28). Arising anteriorly, from the dorsal and ventral lobe of each ganglion, is a short anterior nerve which extends forwards (Text-figs. 7, 28, 29). Each divides into two in a dorso-ventral plane, the division being best seen in sagittal section. One branch, the inner in each case, extends straight forwards to the apex, while the outer pass one to each 'corner' of the apical disk. The whole apical region, therefore, is well innervated. Arising from the posterior region of each ganglion dorsally and ventrally and level with the transverse commissure is a posterior nerve (Text-figs. 28, 29). The four posterior nerves pass slightly obliquely backwards and can be followed for only a short distance. It is probable that these nerves supply the most muscular region of the scolex which lies just behind the nerve centre.

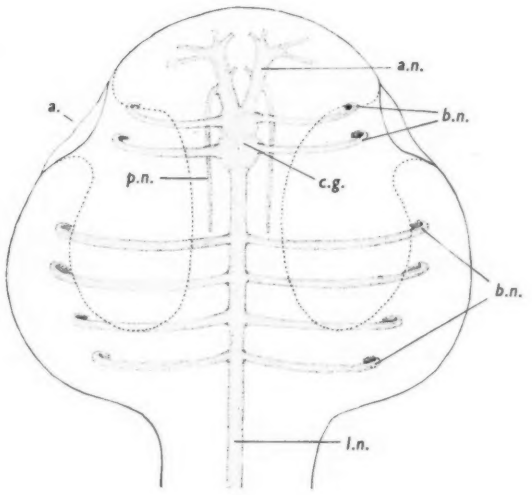
The lateral nerves arise one on either side from the posterior end of the ganglia and pass back through the scolex and on into the strobila (Text-figs. 28, 29). Arising from the lateral nerves in the scolex are the bothrial nerves which supply the bothria. There are eighteen pairs on either side, regularly arranged from a short distance behind the ganglia to the posterior limit of the scolex. They arise dorsally and ventrally, in pairs, from each lateral nerve and take a straight course passing through the longitudinal muscle layer dorsally and ventrally. Just outside the muscle layer each divides into two branches, one turning outwards, slightly, and continuing to the margin of the bothrium and the other inwards towards the deepest part of the bothrial cavity (Text-figs. 3, 28, 29).

This is a very simple arrangment of nerves, but one which is perfectly suited to the musculature in this type of scolex. There are no special apical organs requiring innervation and the bothria are elongated shallow grooves showing slight variation in structure along their length. They are therefore provided with a regular series of nerves repeated from one end to the other. This repetition of bothrial nerves is also seen in *Dibothriorhynchus grossum* (Rud.) (=*Hepatoxylon trichiuri* Holten) where there are twenty-five pairs supplying each of the elongated bothridia (Rees, 1941*a*). The part of the bothrium in *B. scorpii* which is a little more muscular than the rest is provided with an additional nerve, the so-called posterior nerve.

Clestobothrium crassiceps. The nervous system consists fundamentally of almost precisely the same elements as in *B. scorpii*. Cooper (1918) had given a brief account of the nervous system to which more details are added here. The cerebral ganglia are not quite as near the apex of the scolex, relatively, as in *B. scorpii*. They are situated at about one-third of the length of the scolex from the anterior extremity and level with the apertures of the bothria (Text-figs. 30, 31). There is a bilobed ganglion on each side united at their posterior limits by a median transverse commissure and at their anterior limits by a ring-shaped or slightly hexagonal commissure (Text-figs. 16, 18, 30). The two commissures are further apart than in *B. scorpii*. Between the two, but nearer the transverse commissure, is an × -shaped commissure uniting diametrically apposite ganglia and crossing in the middle of the scolex (Text-figs. 17, 30). This does not pass through an aperture in the transverse commissure as in some cestodes, e.g. *Taenia taeniaeformis* (Rees, 1951) and *Tetrabothrius affinis* (Rees, 1956). The × -shaped commissure is not present



30



31

Text-figs. 30, 31. *Clestobothrium crassiceps* (Rud.).

Text-fig. 30. Nervous and excretory system of scolex, ventral view. Bothrial nerves shown on right and excretory system on left.

Text-fig. 31. Nervous system of scolex, lateral view.

in *B. scorpii* and may be associated in some way with the hemispherical type of adhesive organ.

There are again four anterior nerves arising one from the dorsal and ventral lobes of each ganglion (Text-figs. 19, 30, 31). As the ganglia are further back, these nerves are longer than in *B. scorpii*. Passing forwards they diverge from one another dorsally and ventrally and divide again in a dorso-ventral direction, each into several branches which spread out fanwise to supply the anterior extremity (Text-figs. 30, 31). Posterior nerves are again recognizable, but whether they correspond to the posterior nerves of *B. scorpii* is not certain. Each arises from the anterior nerves dorsally and ventrally, and not from the cerebral ganglia. They all turn backwards, immediately, and pass parallel to one another straight into the central zone of the scolex to terminate opposite the posterior margin of the bothrial aperture. It is possible that some additional innervation is necessary where the bothrial wall is incomplete due to the presence of the obliquely placed aperture.

The lateral nerves arise one from each ganglion on either side at the same level as the transverse commissure. They diverge slightly and extend through the remainder of the scolex and into the strobila (Text-figs. 30, 31).

The bothrial nerves are slightly differently arranged in *C. crassiceps*. There are six pairs on each side instead of eighteen, the reduced number corresponding with the reduction in length of the scolex and bothria. The first two pairs arise from the ganglia and not from the lateral nerve cords. The first pair has its origin opposite the ring commissure (Text-figs. 18, 30, 31) and the second opposite the × -shaped commissure (Text-figs. 17, 30, 31). They pass outwards, curving through the apical wall of the bothrium dorsally and ventrally. The remaining four pairs are separated from the first two by a short distance which is occupied by the length of the posterior nerves (Text-figs. 30, 31). They arise regularly in pairs from the lateral nerves in the posterior half of the scolex and curve round in the outer wall of each bothrium below the aperture (Text-fig. 15). Six pairs of bothridial nerves are found, also, in *Aporhynchus norvegicus* (Olss.); three, in this case, arising from the cerebral ganglia and three from the lateral nerve cord on either side (Rees, 1941 *b*). No separate nerves were found supplying the sphincter muscles, presumably they are innervated by branches from the bothrial nerves.

The arrangement of the nervous system, therefore, is fundamentally the same in *B. scorpii* and *C. crassiceps*. Each consists of two bilobed cerebral ganglia united by a transverse and a ring commissure, anterior nerves to the anterior extremity, posterior nerves to the zone behind the commissures, and two lateral nerve cords from which arise bothrial nerves to supply the bothria. The main differences are the presence of an × -shaped commissure in *C. crassiceps* and the reduction in the number of bothrial nerves. This reduction would obviously follow on the reduction in length of the scolex and consequently of the bothria themselves. In those cestodes with an almost spherical scolex and four highly muscular cup-shaped suckers, the bothrial nerves are reduced to from one to about three separate nerves, and not pairs of nerves to each sucker, e.g. *Taenia taeniaeformis* (Rees, 1951) and *Tetrabothrius affinis* (Rees, 1956). The whole nervous system of the scolex in *C. crassiceps* is more concentrated than in *B. scorpii*. In some of the Tetraphyllidea this concen-

tration is even more marked as the central zone of the scolex is greatly reduced in size and the bothridial nerves, which are few in number, arise in almost the same place, e.g. *Anthobothrium auriculatum* (Rud.) (Rees, 1943) and *Phyllobothrium dohrnii* (Oerley) (Rees, 1946). The significance of the × -shaped commissure is difficult to determine, but it exists in the central nervous system of many cestodes. There are no hooks, no rostellum or other apical organs in either *B. scorpii* or *C. crassiceps*, the muscles of which would require a special development of nerves, as, for example, in the case of the rostellum and hook muscles of *Taenia taeniaeformis*. Instead, the apex of the scolex is quite simple in each case, which accounts for the simplicity in the arrangment of the anterior nerves supplying it.

There is, therefore, in the scolex of cestodes a direct relationship between the form of the scolex, the arrangement of the muscles of the various types of adhesive organs, and the arrangement and degree of complexity of the nervous system. This relationship is seen clearly in the two species described here, as it is, also, in scolices of very different form, e.g. *Dibothriorhynchus grossum*, *Phyllobothrium dohrnii* and *Taenia taeniaeformis*.

EXCRETORY SYSTEM OF SCOLEX

The excretory system of the scolex has not been examined in great detail in either *B. scorpii* or *C. crassiceps*, but the arrangement of the main vessels has been followed, some differences noted and these associated, again, with the differences in structure of the scolex.

Bothriocephalus scorpii. The excretory system of the strobila has been very well described by Fraipont (1881). Six longitudinal vessels enter the scolex from the anterior region of the strobila and are arranged in a group of three to the inner side of each lateral nerve cord (Text-figs. 1–3). These continue forwards through the scolex, maintaining the same position but decreasing slightly in diameter, to a short distance behind the nerve centre. Here they all begin to branch, the branches anastomosing with one another on each side. Some pass forwards through the ring commissure and others to the outside of it (Text-fig. 6). In front of the nerve centre the vessels from right and left sides form a general network of fine vessels through the whole of the apical region of the scolex (Text-fig. 7).

The finer branches and superficial network of vessels described by Fraipont (1881) for the strobila have not been found in the scolex, but are probably present.

Clestobothrium crassiceps. In this species there are only two dorsal and two ventral vessels running through the strobila on either side. The dorsal vessel pursues a very sinuous course from the dorsal to the ventral sides of the medulla (Text-fig. 22). In transverse sections, therefore, it may appear either dorsally or ventrally or may even be cut twice in the same section, giving a false impression of a total of six vessels instead of four. The ventral vessel is less tortuous (Text-fig. 21). These vessels enter the scolex to the inner side of the lateral nerves and immediately branch to form a network across the base of the scolex (Text-fig. 30). This network continues outwards dorsally and ventrally, curving around the posterior and outer walls of the bothria. This basal network is not present in *B. scorpii*, presumably because there is no outer bothrial wall in this species. From the central part of the

basal network a single vessel continues forwards on either side through the central zone slightly ventral to the level of the lateral nerve (Text-figs. 14–16, 30). As it passes forwards it may divide and rejoin, but does not appear to give off branches. Near the anterior end and just in front of the nerve centre, the vessel divides into many branches on either side which anastomose with one another to form an apical network as in *B. scorpii* (Text-figs. 17–20, 30). This extends throughout the apical region of the scolex and into the anterior border of the bothria dorsally and ventrally.

There are, therefore, some similarities in the arrangement of the excretory vessels in the scolex of both species and some differences. The main difference is the presence of the basal network of vessels at the posterior end of the scolex in *C. crassiceps* and which is presumably related to the presence of the outer bothrial walls. Following this, there is a reduction in the number of vessels continuing forwards through the central zone of the scolex, but the apical network is present in both.

SUMMARY

1. Two Pseudophyllidea, *Bothriocephalus scorpii* (Müller) and *Clestobothrium crassiceps* (Rud.), have been obtained from *Scophthalmus maximus* (L.) and *Merluccius merluccius* (L.) respectively, from fishing grounds to the west of the British Isles.

2. The musculature of the scolex in both has been compared relative to the form and mode of function of that organ. All the major groups of muscles are present in both, but their arrangement is modified in *C. crassiceps*, due to the presence of hemispherical bothria, instead of open grooves, and a sphincter muscle around the bothrial opening.

3. The mode of attachment is discussed briefly in *C. crassiceps*, and in more detail in *B. scorpii*, where the structure of the scolex was found to be admirably suited to the form of the host's mucosa. The effect on the mucosa is slight, due to the absence of very powerful muscles and of other specialized adhesive structures.

4. The nervous system of the scolex in both species has been compared, and a fundamental similarity has been found. Modifications in *C. crassiceps* are associated with shortening of the scolex and the difference in form of the bothria.

5. The excretory system shows the same correlation with the form of the scolex.

REFERENCES

BAYLIS, H. A. (1939). Further records of parasitic worms from British vertebrates. *Ann. Mag. Nat. Hist.* S. **11**, 4 (23), 473–98.

BELLINGHAM, O'B. (1844). Catalogue of Irish Entozoa, with observations. *Ann. Mag. Nat. Hist.* **13**, various pages.

COOPER, A. R. (1918). North American pseudophyllidean cestodes from fishes. *Illinois Biol. Monogr.* **4** (4), 154–70, 186–200.

DIESING, K. M. (1850). *Systema helminthum* **1**, 692 pp. Vindobonae.

DOLLFUS, R. PH. (1956). Liste des parasites animaux du hareng de l'Atlantique nord et de la Baltique. *J. Cons. Int. Explor. Mer.* **22**, 1, 58–65.

FRAIPONT, J. (1881). Recherches sur l'appareil excréteur des trematodes et des cestodes; deuxième partie. *Arch. Biol., Paris*, **2**, 1–40.

HILMY, I. S. (1939). *Bothriocephalus scorpii* (Mueller 1776), Cooper 1917. *Ann. trop. Med. Parasit.* **23**, 385–96.

HYMAN, L. H. (1951). *The Invertebrates*, vol. 11. McGraw-Hill Book Company, Inc.

JOHNSTONE, J. (1909). Internal parasites and diseased conditions of fishes. *Trans. Biol. Soc. Liverpool*, **32**, 87–100.

JOYEUX, CH. & BAER, J. G. (1936). *Faune de France*, 30, *Cestodes. Paris.* Paul Lechevalier et Fils.

LÖNNBERG, E. (1891). Anatomische Studien über Skandinavische Cestoden. *K. svenska VetenskAkad. Handl.* **24**, 1–109.

MARKOWSKI, S. (1935). O cyklu rozwojowym *Bothriocephalus scorpii* (Müller 1776)..., etc. *Bull. int. Acad. P. Cracovie* (*Acad. pol. Sci.*), (11) (**3/5**), 1–17.

NICOLL, W. (1907). A contribution towards a knowledge of the entozoa of British marine fishes. Part 1. *Ann. Mag. Nat. Hist.* (7), **19**, 66–94.

NIEMIEC, J. (1888). Untersuchungen über das Nervensystem der Cestoden. *Arb. zool. Inst. Univ. Wien*, **7**, 1–60.

REES, G. (1941*a*). The musculature and nervous system of the plerocercoid larva of *Dibothriorhynchus grossum* (Rud.). *Parasitology*, **33**, 373–89.

REES, G. (1941*b*). The scolex of *Aporhynchus norvegicus* (Olss.). *Parasitology*, **33**, 433–8.

REES, G. (1943). The anatomy of *Anthobothrium auriculatum* (Rud.) (Cestoda) from *Raja batis* L. *Parasitology*, **35**, 1–10.

REES, G. (1946). The anatomy of *Phyllobothrium dohrnii* (Oerley) from *Hexanchus griseus* (Gmelin). *Parasitology*, **37**, 163–71.

REES, G. (1951). The anatomy of *Cysticercus taeniae-taeniaeformis* (Batsch, 1786) (*Cysticercus fasciolaris* Rud. 1808), from the liver of *Rattus norvegicus* (Erx.), including an account of spiral torsion in the species and some abnormalities in structure. *Parasitology*, **41**, 46–59.

REES, G. (1956). The scolex of *Tetrabothrius affinis* (Lönnberg), a cestode from *Balaenoptera musculus* L., the Blue Whale. *Parasitology*, **46**, 425–42.

REES, G. & LLEWELLYN, J. (1941). A record of the trematode and cestode parasites of fishes from the Porcupine bank, Irish Atlantic slope and Irish sea. *Parasitology*, **33**, 390–6.

THOMPSON, W. (1844). Additions to the fauna of Ireland. *Ann. Mag. Nat. Hist.* **13**, 430–40.

WARDLE, R. A. (1933). Cestoda of Canadian fishes. 1. The Pacific coast region. *Contr. Canadian Biol.* **7** (18), 221–43.

WARDLE, R. Z. & MCLEOD, J. A. (1952). *The Zoology of Tapeworms.* University of Minnesota Press.

EXPLANATION OF PLATES XVIII AND XIX

Photomicrographs by D. Hemingway Jones, B.Sc., to whom I am most grateful.

PLATE XVIII

Fig. 1. Scolex of *C. crassiceps* showing bothria filled with pieces of mucosa torn from the intestine of the host.

Fig. 2. Sagittal section through scolex of *C. crassiceps* showing sphincter of one bothrium gripping part of the torn mucosa.

Fig. 3. Portion of intestine of *Scophthalmus maximus*, surface view, showing a very heavy infestation with *B. scorpii*.

Fig. 4. Portion of *S. maximus*, showing mucosa raised into primary and secondary folds.

Fig. 5. Portion of intestine of *S. maximus* with several specimens of *B. scorpii* deeply embedded between the primary folds.

PLATE XIX

Fig. 6. Cross-section of the intestine of *Scophthalmus maximus* showing primary and secondary folds and scolices of *B. scorpii in situ*.

Fig. 7. A similar section where the primary folds are deeper. Two 'tunnels' are apparent, occupied each by a scolex of *B. scorpii*.

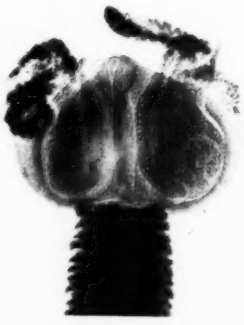
Fig. 8. Portion of a secondary fold showing normal epithelium on right and slight changes in the epithelium where gripped by the bothria of *B. scorpii*.

KEY TO LETTERING OF FIGURES

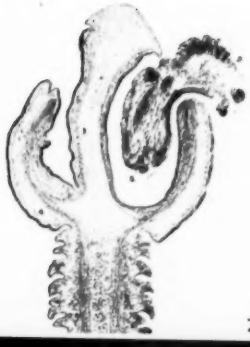
(All drawings are semi-diagrammatic)

a.	aperture of bothrium	*l.m.*	longitudinal muscles
a.n.	anterior nerve	*l.n.*	lateral nerve
a.n.e.	apical network of excretory vessels	*o.t.m.*	outer transverse muscles
b.	bothrium	*p.n.*	posterior nerve
b.n.	bothrial nerve	*r.a.*	radiating apical muscles
b.n.e.	basal network of excretory vessels	*r.c.*	ring commissure
c.g.	cerebral ganglion	*r.m.*	radial muscles
c.m.	circular muscles (outer transverse muscles)	*sp.*	spincter muscle
		t.	testis
d.e.v.	dorsal excretory vessel	*t.c.*	transverse commissure
d.v.m.	dorso-ventral muscles	*tn.*	tendon
d.v.o.	dorso-ventral oblique muscles	*v.*	vitellaria
e.v.	excretory vessel	*v.e.v,*	ventral excretory vessel
i.t.m.	inner transverse muscles	*x.*	× -shaped commissure
l.g.	lateral groove		

(*MS. received for publication* 13. VI. 1957.—Ed.)



1



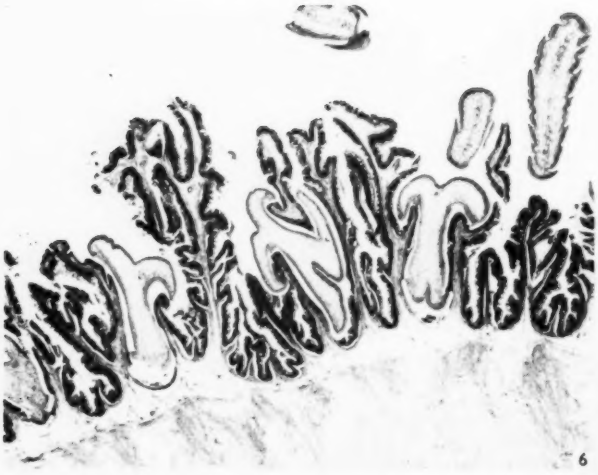
2

3

5

4

(*Facing p.* 492)



THE EPIDEMIOLOGY OF THE INFESTATION OF THE HONEYBEE, *APIS MELLIFERA* L., BY THE MITE *ACARAPIS WOODI* RENNIE AND THE MORTALITY OF INFESTED BEES

By L. BAILEY

Bee Department, Rothamsted Experimental Station

(With 5 Figures in the Text)

INTRODUCTION

Acarapis woodi Rennie, a mite which infests the anterior thoracic tracheae of the honeybee, was discovered and described by Rennie, White & Harvey (1921). They considered the mite to be the cause of a disease of honeybees known as the Isle of Wight disease. Lesions of various kinds, caused by the mite, have been described as occurring in infested bees by Rennie *et al.* (1921), Anderson (1928), Morgenthaler (1944) and Schneider & Brügger (1949).

Morgenthaler (1931*a*) showed that for transmission of the mite to occur intimate bodily contact between bees was necessary; bees separated by a wire gauze, through which they could feed each other, did not transmit mites to each other. Hirschfelder (1952) showed that a mite transfers itself from one bee to another by climbing to the tip of a hair, which it grasps with its hindlegs, and then seizing the hair of any other bee which brushes by, with its forelegs. Morgenthaler also showed (1930, 1931*a*) that only bees less than 12 days old can become infested. His various tabulated results have been plotted together, graphically, in Fig. 1, which makes it clear that susceptibility of bees to infestation diminishes very rapidly from their first day of life. Sachs (1952) found that mites can enter the first thoracic spiracle of young bees only, because the fringe of hairs which guard the spiracle entrance has not had time to become sufficiently stiff.

Some observations by Morgenthaler (1929, 1930, 1932) made it appear that the proportion of infested bees in colonies kept in natural conditions varied during the year. The proportion of infested bees, highest in autumn, fell to a minimum about March and then rose during the summer. Later, however, Morgenthaler (1944) considered the annual fluctuation to be slight. Morison, Jeffree, Murray & Allen (1956) found a marked fluctuation of the mean monthly percentage of samples with infested individuals in samples of bees sent to them for diagnosis in a period of 22 years from various parts of the British Isles. A well-defined minimum occurred about May; the proportion of infested samples then rose quickly during the summer to reach a maximum in August and then began to decline rapidly about February. Morgenthaler and Morison *et al.* found respectively that the percentage of infested bees in colonies and of samples with infested bees remained fairly steady during the winter months.

Jeffree (1955) found a statistically significant correlation between the reported

presence of *Acarapis woodi* in various parts of the world and a certain annual temperature range. He suggested that the mites were absent outside those regions enclosed by July isotherms (actual) of 11° and 19° C. and January isotherms of −3° and 12° C. as bees were active and short-lived in higher temperatures and mites had less chance to mature and migrate.

As no experiments to determine the effect of infestation on the length of life of a bee or of a colony have been reported, and as no further detailed observations and experiments on its epidemiology seem to have been made, these aspects have been studied in recent years in the Bee Department at Rothamsted Experimental Station, Harpenden.

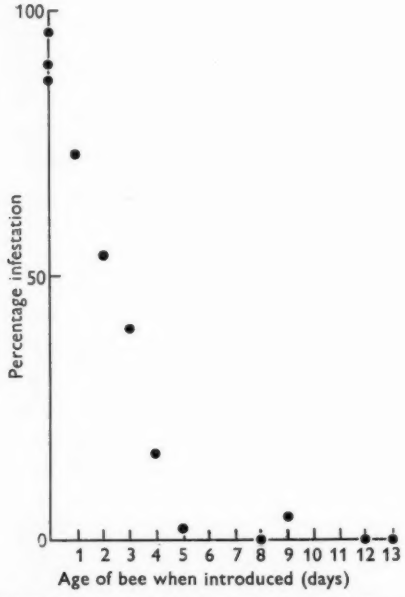


Fig. 1. The proportion of healthy bees of different ages which became infested by *Acarapis woodi* Rennie after they were introduced to populations of infested bees. (After Morgenthaler, 1930, 1931*a*.)

METHODS

The percentage of infested bees was found in samples of 100 bees. Bees were dissected in the way described in the Advisory Leaflet of the Ministry of Agriculture, Fisheries and Food (1950). The degree of infestation of a trachea was classified as nil, light, or heavy; tracheae infested to about half their lengths with mites were considered lightly infested and those completely or nearly so were considered heavily infested. The number of infested bees with mites in both tracheae was usually recorded; it was positively correlated with the number of heavily infested bees but was not found helpful in analyses of the results and is not usually given below. It was generally found that bilaterally infested bees had an equivalent infestation in each trachea; where this was not so, the heaviest infestation was recorded.

Newly emerged bees were obtained by incubating combs of sealed brood at 34–35° C. for 24 hr. or less. Each bee was marked with a spot of quick-drying paint on the dorsal surface of its thorax.

Colonies of bees were kept at sites near Rothamsted Experimental Station, Harpenden.

In laboratory experiments, the bees were kept in small cages made of wood and wire gauze, each of which was supplied with two glass tube feeders, one of which contained concentrated sucrose solution and the other distilled water. Cages were incubated in a dry atmosphere at 30° C. unless otherwise indicated.

EXPERIMENTS AND RESULTS

(*a*) *Field observations*

(1) *Epidemiology*

The percentage of infested bees was found at intervals in three colonies A, B and C over the periods October 1955 to February 1957, October 1956 to September 1957, and May 1957 to September 1957 respectively. Most of the bees of colonies

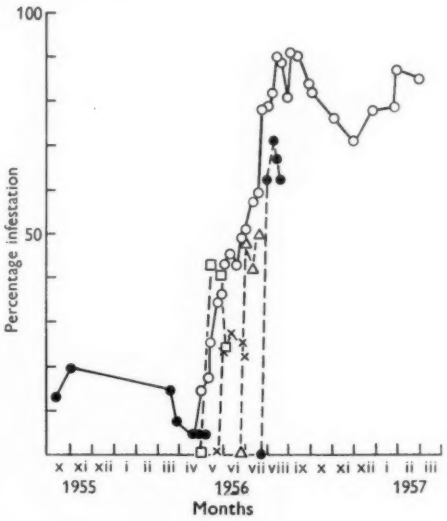


Fig. 2

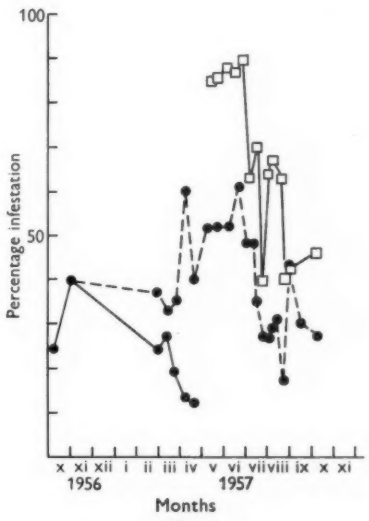


Fig. 3

Fig. 2. The proportion of infested bees in colony A at different times of the year. ●—●, bees marked in October, 1955; ○—○, unmarked bees. □----□, ×----×, △----△, ●----●, young bees which emerged in an incubator and were then introduced to the colony.

Fig. 3. The proportion of infested bees in colonies B and C at different times of the year. ●—●, bees marked in October 1956 (colony B); ●----●, unmarked bees (colony B); □—□, unmarked bees (colony C).

A and B were marked in October 1955 and October 1956 respectively. Newly emerged bees, in groups of about 1500, were marked and introduced into colony A on four occasions during the spring and summer of 1956, and the percentages of infested bees in them were determined at approximately weekly intervals after their introduction. The last samples, taken on 7 June and 4 July, of the first two of these

groups contained only 29 and 49 bees respectively. Colony B was kept on the exact position occupied during 1956 by colony A. Colony C was about half a mile away from colony B. The results are given in Figs. 2 and 3. The proportion of infested bees which were heavily infested in colonies A and B is given in Fig. 4.

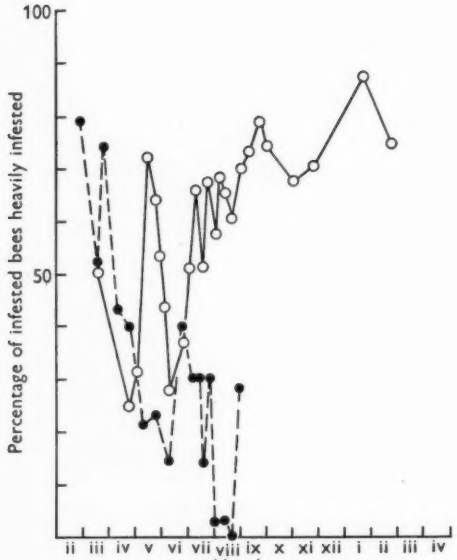


Fig. 4

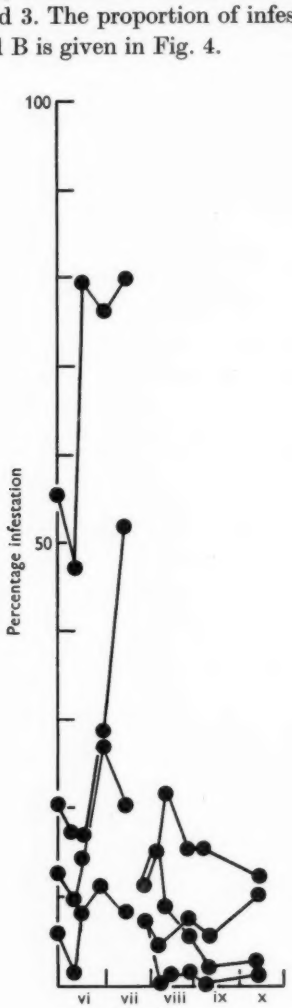


Fig. 5

Fig. 4. The proportion of infested unmarked bees which were heavily infested in colonies A and B. ○—○, colony A (1956); ●----●, colony B (1957).

Fig. 5. The proportion of infested bees in eight colonies during the summer of 1955.

During 1955 the percentage of infested bees was found on several occasions in colonies used as controls for experiments with acaricides (Bailey & Carlisle, 1956). The results are shown in Fig. 5. The records were not continuous; the four colonies whose courses of infestation are shown from July to October were not the same as

those of the June to July period, but they were all the most heavily infested colonies which could be found at the time.

All the colonies of bees at Rothamsted were sampled in the autumn (September–October) of 1951, 1953, 1954, 1956 and 1957, and in the spring (March–April) of 1952, 1953, 1955, 1956 and 1957; samples of 30 bees only were taken from each colony on these occasions. Trials of treatments for reducing infestation of bees with the mite were not carried out during these years except on a very small scale in 1955 and during the winter of 1956–57 and the treated colonies have not been included in the figures given below. The results are given in Table 1.

Table 1. *Infestation of colonies of bees with* Acarapis woodi *at Rothamsted*

		No. of infested bees per sample of 30		
		0	0–3	Over 3
		No. of colonies		
Year	Time			
1951	Autumn	126	17	5
1952	Spring	109	10	2
1953	Spring	110	15	5
1953	Autumn	132	26	16
1954	Autumn	93	17	4
1955	Spring	174	39	12
1956	Spring	251	14	3
1956	Autumn	188	46	26
1957	Spring	172	48	11
1957	Autumn	169	42	32

(*b*) *Laboratory experiments*

(i) The effect of temperature on the transmission of the mite was found by introducing 20 newly emerged marked bees into each of several cages, each of which contained 100 bees taken at random from a heavily infested colony. The bees had abundant space in the cages. Some of the cages were incubated at 23° C, some at 30° C. and some at 35° C., the latter being about the maximum temperature reached in brood nest of colonies in summer. All the marked bees and 100 of the unmarked bees were dissected 6 days later. The results of the experiment, which was done in two separate stages, A and B, at different times of the year, are given in Table 2.

The mean of the percentages of infested young bees in cages 1–3 was significantly less (t test, $P < 0{\cdot}01$) than the percentage of infested young bees in cage 4. If it is assumed that any (unknown) variations between cages at the same temperatures did not affect the migration of mites, then the proportion of infested individuals amongst the young bees was significantly greater (χ^2 analysis, $P < 0{\cdot}05$) in cages $7+8$ than in cages $5+6$.

(ii) A greater proportion of old infested bees in Expt. A were more heavily infested than in Expt. B (χ^2 analysis, $P < 0{\cdot}001$) and there seemed to be a higher proportion of infested bees amongst the young bees in cage 4 than in cages $5+6$ (Table 2); the difference was hardly significant however ($P < 0{\cdot}06$). As the experi-

ments had been carried out at different times, which might have influenced the result, a further experiment was made to find the effect of the degree of infestation of old bees upon the proportion of young bees which becomes infested. Two thousand newly emerged bees were marked and introduced into a heavily infested colony on 1 May 1957. On 29 May, 200 of the marked bees were recovered off the combs in the hive and 100 of them were placed in each of two cages (nos. 3 and 4). One hundred unmarked bees, taken at the same time off the combs in the same hive,

Table 2. *The infestation by* Acarapis woodi *of young healthy bees caged with old infested bees at different temperatures after 6 days*

Expt. A (13 March 1957)

Infestation of old bees

Not infested	Lightly infested	Heavily infested
37	23	40

Infestation of young bees

Cage	Temp. (° C.)	Not infested	Infested
1	23	15	3
2	23	18	2
3	23	12	2
4	30	7	11

Expt. B (14 August 1957)

Infestation of old bees

Not infested	Lightly infested	Heavily infested
42	44	14

Infestation of young bees

Cage	Temp. (° C.)	Not infested	Infested
5	30	12	7
6	30	15	5
7	35	8	12
8	35	10	10

were placed in each of two more cages (nos. 1 and 2). (As the average length of life of bees at this time of the year is probably no more than 5 weeks (Ribbands, 1953), it is likely that the age of the unmarked bees was rather less than that of the marked bees.) Twenty newly emerged, distinctively marked, bees were then placed in each of the four cages. The bees were dissected 6 days later. The results are given in Table 3.

The proportion of heavily infested bees in the old infested bees was higher in cages 1 and 2 than in cages 3 and 4 (χ^2 analysis, $P < 0{\cdot}01$), and the percentage of infested bees in the young bees in cages 1 and 2 was greater than in cages 3 and 4 (t test, $P < 0{\cdot}02$).

Table 3. *The infestation of young healthy bees caged with old bees with different degrees of infestation, at* 30° C. *after* 6 *days*

	Infestation of young bees		Infestation of old bees		
Cage	Not infested	Infested	Not infested	Lightly infested	Heavily infested
1	10	10	28	39	29
2	7	11			
3	18	2	26	58	16
4	17	3			

(2) *The mortality of infested bees*

(*a*) *Field observations*

(i) The mortality of infested bees can be deduced from Figs. 2 and 3. The percentage of infested individuals in the bees marked in autumn in colonies A and B fell significantly (χ^2 analysis, $P < 0{\cdot}001$) from the winter level during March and April of 1956 and 1957. Thus, the infested bees were dying sooner than the non-infested bees of the same age. This was also suggested by the lightness of infestation of the surviving infested bees: only one infested marked bee out of 18 had a heavy infestation in the March to May period in colony A; 43 infested unmarked bees out of 73 from the same hive had heavy infestation in the same period. But all the marked bees were dying quickly at the same time: a marked reduction in numbers of marked bees was seen in colony A in mid-March; about 12 were found in mid-May and two or three only at the end of May. Similarly, in colony B, it was difficult to find 100 marked bees towards the end of April and only one marked bee was seen at the beginning of May. The increased mortality due to mite infestation was, therefore, only slight, although significant. The fall of the percentages of infested bees in the last samples from the highest levels reached in three of the four groups of newly emerged marked bees introduced into colony A in 1956 (Fig. 2) were not statistically significant; but as three of the groups out of four showed a fall, it may have been a real trend.

(ii) Samples of flying bees from infested colonies were always found to contain heavily infested individuals; an example is given in Table 4.

Table 4. *The infestation of flying bees and bees from within the hive of an infested colony on* 15 *May* 1957

	Lightly infested	Heavily infested	Bilaterally infested
Flying bees	40	39	41
Bees from within the hive	60	25	38

It was not possible to draw conclusions from a comparison of infestation of flying bees and household bees at the time as the two groups would not have been from populations of the same age. Nevertheless, the normal activity of flying bees did not give any impression that they were affected by their heavy infestation.

On one occasion some of the bees of a heavily infested stock were reported to

be crawling outside their hive in February 1957 (crawling of bees was one of the more generally reported symptoms of the Isle of Wight disease), but the proportion of infested bees (22 of a sample of 28 bees) was not significantly higher than that of the household bees (57 out of 100) which appeared normal and were probably about the same age at the time.

(iii) The mortality of infested colonies during the winter of 1956–57 is shown in Table 5.

Table 5. *The mortality of colonies of bees, infested and non-infested with* Acarapis woodi, *at Rothamsted during the winter* 1956–57

No. of infested bees per 30 bees (autumn 1956)	No. of colonies	No. which died
0	188	10
1– 9	57	0
10–20	6	2
21–25	3	1
26–30	3	3

(*b*) *Laboratory experiments*

The mortality of bees infested with *Acarapis woodi* and of similarly infested bees which were also infected with *Nosema apis* Zander, were compared with that of non-infested bees as follows. Twenty-five bees from a colony with 50% of its bees infested with *Acarapis woodi*, but free from *Nosema apis*, were placed in each of 22 cages. The bees in 12 of these cages were artificially infected with *N. apis* by feeding them a suspension of spores in the way described by Bailey (1955). Dead bees were removed daily from the cages and examined for infestation with *Acarapis woodi*. On the day when 13 or more bees had died in a cage, the remaining live bees in it were dissected.

Table 6*a*. *Mortality of caged bees infested with* Acarapis woodi *and infected with* Nosema apis

	Half-lives (days) of cage populations of 25 bees	
	Bees infested with *A. woodi* + *N. apis*	Bees infested with *A. woodi* only
	18	16
	18	26
	18	26
	18	26
	21	29
	22	29
	22	30
	22	30
	22	30
	22	34
	24	—
	24	—
Mean half-life	20·9	27·6

These means are significantly different (t test, $P < 0{\cdot}001$)

Table 6*b*

	Bees not infected with *N. apis*			Bees infected with *N. apis*		
	Not infested with *A. woodi*	Infested with *A. woodi*		Not infested with *A. woodi*	Infested with *A. woodi*	
		Lightly	Heavily		Lightly	Heavily
Total no. of bees from all cages which died in half-life periods	70	28	57	98	38	75
Remainder	40	15	24	45	22	16

The durations, to the nearest day, of the life of half the population (its half-life) in each of the 12 cages in which the bees had been infected with *Nosema apis* were compared with those in the other 10 cages. The results are given in Table 6(*a*).

A comparison of the proportion of bees infested and not infested with *Acarapis woodi* was made, within the two groups infected and not infected with *Nosema apis*, between those bees which had died during the half-life period and the remainder. The results are given in Table 6(*b*).

There was no significant difference between the proportion of bees infested with *Acarapis woodi* which died in the half-life periods and that in the remainder, neither of those bees infected with *Nosema apis* nor of those not so infected.

The proportion of heavily infested individuals, in the bees infested with *Acarapis woodi* and infected with *Nosema apis*, which died during the half-life periods was significantly higher (χ^2 analysis, $P < 0{\cdot}01$) than that in the remainder.

DISCUSSION

(1) *Epidemiology*

The results shown in Figs. 2 and 3 make it clear that the variation of the proportion of honeybees infested by *Acarapis woodi* is not always the same each year; a striking difference can also occur between the degree of infestation of infested bees in different years (Fig. 4). As only young bees, in intimate contact with infested bees, can become infested, it seems likely that variations in the degree of this contact is the reason for the observed variations of infestation. Thus the epidemic in colony A in 1956 (Fig. 2) was probably due to the lack of foraging activity of the bees in a season in which there was a very poor nectar-flow. During periods of foraging activity the older, more heavily infested bees will contact newly emerged bees less frequently than during periods of no nectar-flow; hence the fall of the percentages of infested bees in colonies B and C (Fig. 3) during the only appreciable nectar-flow of 1957 which began in the latter half of June and continued into the first half of July. The proportion of infested individuals in the second group of young bees introduced into colony A at the end of May 1956 was low; there was a minor nectar-flow during and shortly after their time of introduction. The duration of foraging activity of the older bees need not be long to have a significant effect on the infestations of the younger bees, as the susceptibility of the latter falls very

quickly immediately after they have emerged. The major variations of the percentage of infested bees in colonies B and C during the summer were too great to have been sampling errors; they were probably the effect of short periods of foraging activity followed by short periods of inactivity in a season which had generally variable weather.

The percentages of infested bees in the last three groups of young bees which were introduced into colony A were lower than those of the samples of unmarked bees taken from the colony at the same times; as most of the bees were some hours old when introduced their susceptibility had begun to fall before their introduction. But it may be expected that few newly emerged bees introduced to an infested population would become more infested than large numbers introduced to the same population and this probably accounts for the relatively high proportion of bees infested in the first group of young bees introduced at the end of April before the colony had reached its peak of brood-rearing. The rise of the proportion of infested bees amongst the unmarked bees in colonies A and B during the spring may be accounted for in the same way. During a season with poor nectar-flows this trend of heavy infestation may continue, as it did in colony A which was kept on a site on which colonies of bees gain little honey even in normal years. In more normal seasons, however, even though the proportion of infested bees in the colony may rise, as in colony B during the period March to June (Fig. 3), the degree of infestation of the infested bees may fall when newly emerged bees are becoming numerous (colony B, Fig. 4). As lightly infested bees transmit fewer mites than heavily infested ones (Table 3), the effect of foraging activity of bees in nectar-flows, even though slight, could then be sufficient to lower the proportion of bees which become infested. Infested bees under 14 days old are probably not infestive as the female mite takes this length of time to reach maturity (Morgenthaler, 1931*a*); bees may usually begin foraging at any age from about 9 days (Ribbands, 1952) and can begin at the age of 2 or 3 days (Sakagami, 1953; Simpson, 1950); a good nectar-flow will, therefore, cause a large proportion, at least, of the infestive bees to be occupied in the field.

The short life of the bee in summer does not seem to be the fundamental reason for the lack of infestation of bees in those parts of the world with the warmer summers indicated by Jeffree (1955). The infestation experiments described above (Table 3) showed that bees could transmit mites when they were 4 weeks old, perhaps younger, and the average length of life of bees in the summer is about 5 weeks. Moreover, higher temperatures increase the effective migration of mites between bees (Table 2). The differences between the mean monthly temperatures of the years 1956 and 1957 were very small and did not seem likely to have caused the difference between the course of infestation in colonies A and B (Figs. 2, 3). Furthermore, heavy infestations of *Apis indica* with a mite apparently identical with *Acarapis woodi* were found in 1956 near Simla in India (Milne, 1957); the annual range of the mean monthly temperatures (actual) in the locality is about 6–22° C. and is outside the range of isotherms described by Jeffree as containing those areas with potential infestation. But, to quote information kindly provided by Dr Sarder Singh (Entomologist, Punjab): 'Usually there are two [nectar] flows; spring from Barberry and autumn from *Plectranthus*. Barberry flow is erratic.

Flow from *Plectranthus* was fairly regular previously, but in recent years there have been continuous failures.' It would appear that the heavy infestation of the Indian bees was the effect of the depression of foraging activity of the bees by the failures of the nectar-flow.

The effect of the outstandingly good nectar-flow during 1955 and the almost total absence of one in 1956 is seen in the records of infested colonies at Rothamsted (Table 1). The drop in the proportion of infested colonies in spring 1956 from that in spring 1955 was very significant ($P < 0{\cdot}001$). It might be supposed that this could have been caused by the deaths of the heavily infested colonies during the winter of 1955–56 but, as discussed below, winter losses of infested colonies usually seem small; furthermore, none of the differences between the proportions of infested colonies in the autumn and spring of 1951–52, 1954–55 or 1956–57 are significant so it is very likely that the low proportion of infested colonies in the spring of 1956 was about the same as in the autumn of 1955 and was the result of the exceptional nectar-flow of 1955. The major nectar-flow for the colonies whose percentages of infested bees are given in Fig. 5 was in July 1955 when a marked drop in the percentage of infested bees seems to have occurred. The rise of the proportion of all infested colonies at Rothamsted in autumn 1956 (or in spring 1957) over that in spring 1956 was very significant ($P < 0{\cdot}001$). This coincided with the extraordinary increase of the proportion of infested bees in colony A and was most likely due to the lack of foraging activity of bees in the extremely poor season. There was no significant difference between the proportion of infested colonies in the spring and autumn of 1957; this paralleled the infestation in colony B and was probably typical of an average season.

It seems very likely that the mite is present, or is likely to occur, in regions of the world which have unreliable nectar-flows; many, perhaps most, of such regions may coincide with those within the January and July isotherms found by Jeffree.

The steep rise of the percentage of samples with infested bees received during the summer by Morison *et al.* (1956) may have been due to a tendency for beekeepers to send in more samples during poor summers, when their bees were not doing well, than in good summers. That many such samples contained infested bees would not have detracted from the general belief that the mite was responsible for the poor performance of the colonies.

(2) *The mortality of infested bees*

Rennie *et al.* (1921) considered *Acarapis woodi* to be the cause of Isle of Wight disease of bees, but, as indicated by Bullamore (1922), their evidence was inadequate. They demonstrated that infestation was common in bees, but they did not demonstrate increased mortality of bees caused by the mite. It is possible that bees suffered heavier losses than is usual in the British Isles from the beginning of this century until about 1920, but there is no evidence that the losses were caused by any one known parasite of honeybees. Rennie (1923) said later that 'Under the original and now quite properly discarded designation "Isle of Wight Disease", were included several maladies having analogous superficial symptoms'. Nevertheless, he remained convinced that the mite was very dangerous and his original

claim that it caused the Isle of Wight disease is still generally accepted (e.g. Steinhaus, 1949; Cameron, 1956; Snodgrass, 1956). But infestation with *A. woodi* shortens the lives of bees only slightly, as shown by the field observations described above, and no signs of distress were noticed in infested flying bees or in any infested colony in the summer and autumn during close observations in 1956 and 1957; colony B had gained about 60 lb. of honey by September 1957; this was above average when compared with the performance of non-infested colonies at the same site. When the crawling bees, which did not have a significantly different infestation from bees within the hive, were collected (Table 4), bees were found in a similar state outside other hives of colonies known to be free from infestation; it seems likely that the crawling bees are often those which happen to alight on the ground and become chilled; it is a common phenomenon on bright days with a low shade temperature.

It has been suggested that bees to-day are less susceptible to infestation with the mite than in the days of the Isle of Wight disease (Anderson, 1930); the resistant bees were thought to have been selected out by the epidemics. If this is so, it seems likely that the selection occurred before the investigations of Rennie *et al.* (1921) as they found heavily infested bees foraging and generally behaving normally; yet the symptoms they described of distressed bees from the same colonies were typical of those of the Isle of Wight disease. They stated that 'Egyptian, Dutch, Punic and Italian bees can be readily infected and in these *Tarsonemus* [*Acarapis woodi*] multiplies with disastrous results, as in British bees'. It seems very likely that the infested colonies were distressed by other unidentified causes, additional to infestation with the mite: such colonies, prevented by some reason from foraging normally or from producing a normal amount of brood might, if lightly infested, have developed their high proportion of infested bees in the ways discussed above; the infestation would then have been the effect of distress, not the cause, although when it became heavy, it might have had a supplementary effect. A great deal of the infestation found by Rennie *et al.* may have been due to poor weather; they made repeated references to the poor season and the lack of surplus honey in the colonies in the year that they made most of their investigations.

The results of the cage experiments described above (Table 5) show no shortening of the lives of infested bees over a period of 3 or 4 weeks. But superimposed infection with *Nosema apis* shortened the lives of heavily infested bees. Mortality of infested bees may be increased when bees are exposed to prolonged unfavourable conditions; e.g. after their long life in the winter cluster, or after the use in autumn of acaracides which are slightly toxic to bees and which led to an earlier death of the infested bees during the winter (Bailey & Carlisle, 1956); effects of the same acaricides were not evident over short periods of time (Morgenthaler, 1931*b*). All this is in accord with lack of visible signs of infestation of colonies during the summer as bees then normally live for 5 weeks or thereabouts, and again suggests that the Isle of Wight disease, symptoms of which appeared during the summer and autumn as well as the spring, was due to factors other than, or supplementary to, infestation with the mite. It appears from the number and infestation of colonies which died at Rothamsted during the winter of 1956–57 (Table 5) that

chances of death of colonies with over about 30 or 40% of their bees infested are increased; but even in the worst season, typified by that of 1956, the proportion of infested bees in most colonies remained below about 10% (Table 1). Relatively few colonies, though infested, suffered the inexorable rise of infestation observed in colony A. It is conceivable that a general heavy infestation of the majority of honeybee colonies could build up over a period of years which have consistently bad seasons and that considerable losses would then occur in the final winter, but the likelihood seems small. Such a phenomenon would probably never arise in wild colonies of honeybees; the poor seasons necessary to produce heavy infestation with the mite would probably eliminate most of the colonies by starvation. Infestation of beekeepers' colonies may be high relative to that in wild colonies and, if so, it is the result of keeping alive those colonies, which otherwise would have died of starvation, by feeding them with sugar syrup. Nevertheless, as the records of the colonies and the observations made at Rothamsted show, such colonies appear to suffer little damage from infestation with the mite alone.

SUMMARY

The proportion of young honeybees which became infested with *Acarapis woodi* Rennie rose with an increased degree of infestation of the older bees and increased temperatures. But the most important influence controlling infestation seems to be the frequency with which young and old bees come into contact; this is probably determined by the foraging activity of the bees and the ratio of young bees to old bees; the percentage of infested bees in colonies diminished rapidly during nectar-flows and rose in periods of little nectar-flow.

The mortality of infested bees was only slightly greater than that of non-infested bees and became evident only after the bees had been in prolonged unfavourable circumstances. There were no obvious signs of disease in heavily infested colonies in summer. Colonies with more than about 30 or 40% of infested bees seemed more likely to die in winter than non-infested colonies, but few colonies became so heavily infested even after a very poor season.

This investigation was made possible by the assistance of Mrs E. Booth (née Carlisle) to whom I am very grateful. Thanks are expressed to Dr C. G. Butler and Dr J. Simpson for their helpful criticisms.

REFERENCES

Anderson, E. J. (1928). The pathological changes in honeybees infested with the Isle of Wight Disease. *J. Econ. Ent.* **21**, 404–8.

Anderson, E. J. (1930). Isle of Wight disease in bees. *Bee World*, **11**, 37–42, 50–3.

Bailey L. (1955). The infection of the ventriculus of the adult honeybee by *Nosema apis* (Zander). *Parasitology*, **45**, 86–94.

Bailey, L. & Carlisle, E. (1956). Tests with Acaricides on *Acarapis woodi* (Rennie). *Bee World*, **37**, 85–94.

Bullamore, G. W. (1922). *Nosema apis* and *Acarapis* (*Tarsonemus*) *woodi* in relation to the Isle of Wight bee disease. *Parasitology*, **14**, 53–62.

Cameron, T. W. M. (1956). *Parasites and Parasitism*. London: Methuen.

HIRSCHFELDER, H. (1952). Das Verhalten der Tracheenmilbe *Acarapis woodi* R. beim Verlassen und Aufsuchen der Wirtsbienen. *Z. Bienenforsch.* **1**, 141–7.

JEFFREE, E. P. (1955). Acarine disease of the honeybee and temperature. *Nature, Lond.*, **175**, 91.

MILNE, P. S. (1957). Acarine disease in *Apis indica*. *Bee World*, **38**, 156.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD, ENGLAND AND WALES. (1950). The examination of bees for Acarine disease. *Adv. Leafl. Min. Agric. Fish. Lond.* n. 362.

MORGENTHALER, O. (1929). Problems of Acarine disease of bees. *Bee World*, **10**, 19–24.

MORGENTHALER, O. (1930). New investigations on Acarine disease. *Bee World*, **11**, 49–50.

MORGENTHALER, O. (1931*a*). An acarine disease experimental apiary in the Bernese Lake District and some of the results obtained there. *Bee World*, **12**, 8–10.

MORGENTHALER, O. (1931*b*). Der Sieg über die Bienenmilbe. *Schweiz Bienenztg*, **54**, 498–505.

MORGENTHALER, O. (1932). Ein Jahrzehnt Milbenkrankheit der Honigbiene. Entdeckung, Erforschung und Bekämpfung eines Seuchen-Erregers. *Z. Angew. Ent.* **19**, 449–489.

MORGENTHALER, O. (1944). Das jahreszeitliche Auftreten der Bienenseuchen. *Beih. Schweiz. Bienenztg*, **1**, 285–336.

MORISON, G. D., JEFFREE, E. P., MURRAY, L. & ALLEN, M. D. (1956). Acarine and *Nosema* diseases of honeybees in Britain, 1925–1947. *Bull. Ent. Res.* **46**, 753–9.

RENNIE, J., WHITE, P. B. & HARVEY, E. J. (1921). Isle of Wight Disease in hive bees. *Trans. Roy. Soc. Edinb.* **52**, 737–779.

RENNIE, J. (1923). Acarine disease explained. *Mem. N. Scot. agric. Coll.* no. 6.

RIBBANDS, C. R. (1952). Division of labour in the honeybee community. *Proc. Roy. Soc.* B, **140**, 32–43.

RIBBANDS, C. R. (1953). *The Behavior and Social Life of Honeybees*. London: Bee Research Association Limited.

SACHS, H. (1952). Über das Verhalten und die Orientierung der Tracheenmilbe *Acarapis woodi* (Rennie 1921) auf Bienen. *Z. Beinenforsch.* **1**, 148–70.

SAKAGAMI, S. F. (1953). Untersuchungen über die Arbeitsteilung in einem Zwergvolk der Honigbiene. Beiträge zur Biologie des Bienenvolkes, *Apis mellifera* L. *Jap. J. Zool.* **11**, 117–85.

SCHNEIDER, H. & BRÜGGER, A. (1949). Comment peut-on diagnostiquer l'acariose sans microscope? *Bull. Soc. romande Apic.* **46**, 372–5.

SIMPSON, J. (1950). Unpublished observation.

SNODGRASS, R. E. (1956). *Anatomy of the Honey Bee*. New York: Comstock.

STEINHAUS, E. A. (1949). *Principles of Insect Pathology*. New York: McGraw-Hill.

(*MS. received for publication* 27. x. 1957.—Ed.)

INDEX OF AUTHORS

ANDRADE MEDONÇA, I. DE *See* MEYER, H., OLIVEIRA MUSACCHIO, M. DE and ANDRADE MEDONÇA, I. DE

ARTHUR, D. R. New species of *Ixodes* ticks from eastern Africa, with a description of the male and nymph of *Ixodes oldi* Nuttall, 1913. (With 91 Figures in the Text), 38

ARTHUR, D. R. A review of some ticks (Acarina: Ixodidae) of sea birds, including a consideration of other cognate material. I. (With 13 Figures in the Text), 124

BAILEY, L. The epidemiology of the infestation of the honeybee, *Apis mellifera* L., by the mite *Acarapis woodi* Rennie and the mortality of infested bees. (With 5 Figures in the Text), 493

BELL, E. J. and SMYTH, J. D. Cytological and histochemical criteria for evaluating development of trematodes and pseudophyllidean cestodes *in vivo* and *in vitro*. (With 2 Figures in the Text), 131

BIRD, ALAN F. Further observations on the structure of nematode cuticle. (With Plates VII and VIII), 32

BISHOP, ANN. An analysis of the development of resistance to metachloridine in clones of *Plasmodium gallinaceum*, 210

CLARKSON, M. J. Life history and pathogenicity of *Eimeria adenoeides* Moore & Brown, 1951, in the turkey poult. (With 6 Figures in the Text), 70

CROFTON, H. D. Nematode parasite populations in sheep on lowland farms. IV. The effects of anthelmintic treatment. (With 7 Figures in the Text), 235

CROFTON, H. D. Nematode parasite populations in sheep on lowland farms. V. Further observations on the post-parturient rise and a discussion of its significance. (With 6 Figures in the Text), 243

CROFTON, H. D. Nematode parasite populations in sheep on lowland farms. VI. Sheep behaviour and nematode infections. (With 2 Figures in the Text), 251

DASGUPTA, BISWAPATI. A new schizogregarine, *Mattesia orchopiae* n.sp., in a flea of squirrels in England. (With 14 Figures in the Text), 375

DASGUPTA, B. and MEEDENIYA, KAMANI. The vector of *Hepatozoon sciuri*. (With 7 Figures in the Text), 419

ERASMUS, DAVID A. Studies on the morphology, biology and development of a strigeid cercaria (*Cercaria X* Baylis 1930). (With Plate XIII and 6 Figures in the Text), 312

FOSTER, R. The effects of trematode metacercariae (Brachylaemidae) on the slugs *Milax sowerbii* Férussac and *Agriolimax reticulatus* Müller. (With Plate X), 261

FOSTER, R. Infestation of the slugs *Milax sowerbii* Férussac and *Agriolimax reticulatus* Müller by trematode metacercariae (Brachylaemidae). (With 2 Figures in the Text), 303

GRESSON, R. A. R. The gametogenesis of the digenetic trematode *Sphaerostoma bramae* (Müller) Lühe. (With Plates XI and XII), 293

INGLIS, WILLIAM G. A revision of the nematode genus *Meteterakis* Karve, 1930. (With 22 Figures in the Text), 9

JACK, K. M. Notes on three species of *Pterygosoma* (Acarina: Pterygosomidae) from Gerrhosaurid lizards. (With 3 Figures in the Text), 89

JAIN, G. P. On the egg and miracidium of *Paryphostomum mehrai* (Faruqui). (With 3 Figures in the Text), 96

JAIN, G. P. On *Cercaria mehrai* Faruqui, 1930 with notes on its life-history. (With 4 Figures in the Text), 413

JOYNER, L. P. Experimental *Eimeria mitis* infections in chickens. (With 6 Figures in the Text), 101

LEE, D. L. A note on a species of *Syngamus* found in the intestine of *Turdus pilaris*. (With 5 Figures in the Text), 121

LEE, D. L. On the morphology of the male, female and fourth-stage larva (female) of *Hammerschmidtiella diesingi* (Hammerschmidt), a nematode parasitic in cockroaches. (With 8 Figures in the Text), 433

LEE, D. L. Digestion in *Leidynema appendiculata* (Leidy, 1805), a nematode parasitic in cockroaches. (With 4 Figures in the Text), 437

MCCONNACHIE, ELSPETH W. Factors affecting the growth and behaviour of axenic strains of *Entamoeba invadens* Rodhain, 1934, 423

MEEDENIYA, KAMANI. *See* DASGUPTA, B. and MEEDENYIA, KAMANI.

MEYER, H., OLIVEIRA MUSACCHIO, M. DE and ANDRADE MENDONÇA, I. DE. Electron microscopic study of *Trypanosoma cruzi* in thin sections of infected tissue cultures and of blood-agar forms. (With Plates I–VI), 1

OLIVEIRA MUSACCHIO, M. DE. *See* MEYER H., OLIVEIRA MUSACCHIO, M. DE and ANDRADE MEDONÇA, I. DE.

PATERSON, NELLIE F. External features and life cycle of *Cucumaricola notabilis* nov. gen. et sp., a copepod parasite of the holothurian, *Cucumaria*. (With 11 Figures in the Text), 269

REES, GWENDOLEN. A comparison of the structure of the scolex of *Bothriocephalus scorpii* (Müller 1776) and *Clestobothrium crassiceps* (Rud. 1819) and the mode of attachment of the scolex to the intestines of the host. (With Plates XVIII and XIX and 31 Figures in the Text), 468

ROTHSCHILD, MIRIAM. The bird fleas of Fair Isle. (With 10 Figures in the Text), 382

SMITH, KENNETH M. A study of the early stages of infection with the tipula iridescent virus. (With Plates XIV–XVII), 459

SMYTH, J. D. *See* BELL, E. J. and SMYTH, J. D.

SOLIMAN, K. N. *Globidium gilruthi* (Chatton, 1910) infection in the digestive tract of sheep and goats in Egypt, 291

SPRENT, J. F. A. Observations on the development of *Toxocara canis* (Werner, 1782) in the dog. (With Plate IX and 19 Figures in the Text), 184

TATCHELL, R. J. The physiology of digestion in the larvae of the horse bot-fly, *Gasterophilus intestinalis* (de Geer). (With 8 Figures in the Text), 448

TETLEY, J. H. The sheep ked, *Melophagus ovinus* L. I. Dissemination potential, 353

TETLEY, J. H. The sheep ked, *Melophagus ovinus* L. II. Keds acquired by a lamb from its mother. (With 5 Figures in the Text), 364

THOMAS, J. D. Studies on *Crepidostomum metoecus* (Braun) and *C. farionis* (Müller), parasitic in *Salmo trutta* L. and *S. salar* L. in Britain. (With 10 Figures in the Text), 336

THURSTON, JUNE P. The oxygen uptake of *Trypanosoma lewisi* and *Trypanosoma equiperdum*, with especial reference to oxygen consumption in the presence of amino-acids. (With 7 Figures in the Text), 149

THURSTON, JUNE P. The effect of some metabolic inhibitors on the oxygen uptake of *Trypanosoma lewisi* and *Trypanosoma equiperdum*. (With 14 Figures in the Text), 165

THURSTON, JUNE P. The effect of immune sera on the respiration of *Trypanosoma brucei in vitro*. (With 2 Figures in the Text), 463

WALTERS, V. ANN. Structure, hatching and size variation of the spores in a species of *Nosema* (Microsporidia) found in *Hyalophora cecropia* (Lepidoptera). (With 1 Figure in the Text), 113

INDEX OF SUBJECTS

Acarapis woodi Rennie, epidemiology of infestation of the honeybee, *Apis mellifera* L. by, and the mortality of infested bees, 493
Agriolimax reticulatus Müller, effects of metacercariae of Brachylaemidae on, 261
infestation of, with metacercariae of Brachylaemidae, 303
Anthelmintic treatment, effect of, on nematode parasites of sheep on lowland farms, 235
Apis mellifera L., epidemiology of infestation of, by *Acarapis woodi* Rennie, and mortality of infested bees, 493

Birds, of Fair Isle, fleas of, 382
Bot-fly, digestion in the larvae of, 448
Bothriocephalus scorpii (Müller, 1776), comparison of the scolex and its attachment with those of *Clestobothrium crassiceps* (Rud. 1819), 468
Brachylaemidae, effects of metacercariae of, on the slugs *Milax sowerbii* Férussac and *Agriolimax reticulatus* Müller, 261
metacercariae of, infesting *Milax sowerbii* Férussac and *Agriolimax reticulatus* Müller, 303

Cercaria, mehrai Faruqui, 1930, structure and life-history of, 413
Cercaria, X Baylis, 1930, morphology, biology and development of, 312
Cestodes, pseudophyllidean, cytological and histochemical criteria for evaluating development of, *in vivo* and *in vitro*, 131
Chickens, experimental infection of, with *Eimeria mitis*, 101
Clestobothrium crassiceps (Rud. 1819), comparison of the scolex and its attachment with those of *Bothriocephalus scorpii* (Müller, 1776), 468
Cockroaches, *Hammerschmidtiella diesingi* (Hammerschmidt), a nematode parasitic in, 433
Leidynema appendiculata (Leidy, 1850), a nematode parasitic in, 437
Crepidostomum, farionis (Müller), parasitic in *Salmo trutta* L. and *Salmo salar* L. in Britain, studies on, 336
metoecus (Braun), parasitic in *Salmo trutta* L. in Britain, studies on, 336
Cucumaria, host of *Cucumaricola notabilis*, nov. gen. et sp., 269
Cucumaricola notabilis nov. gen. et sp., parasitic on *Cucumaria*, external features and life cycle of, 269

Digestion, in the nematode *Leidynema appendiculata* (Leidy, 1850), 437
in the larvae of the horse bot-fly, *Gasterophilus intesinalis* (de Geer), 448
Diomedea chlororhynchus, host of *Ixodes diomedeae* n.sp., 124
Dog, development of *Toxocara canis* in, 184

Eimeria, adenoeides Moore & Brown, 1951, life-history and pathogenicity of, in the turkey poult, 70
Eimeria mitis, experimental infection of chickens with, 101
Entamoeba invadens, factors affecting the growth and behaviour of axenic strains of, 423

Fleas, of squirrels in England (*Orchopeas wickhami*), as hosts of the schizogregarine, *Mattesia orchopiae* n.sp., 375
as vectors of *Hepatozoon sciuri*, 419
Fratercula arctica grabae, description of the male of *Ixodes rothschildi* from the burrows of, 124

Gasterophilus intestinalis (de Geer), digestion in the larvae of, 448
Gerrhosaurid lizards, notes on three species of *Pterygosoma* (Acarina: Pterygosomidae) from, 89
Globidium gilruthi (Chatton, 1910), infection of Egyptian sheep and goats with, 291

Hammerschmidtiella diesingi (Hammerschmidt), morphology of the male, female and female fourth-stage larva of, 433
Hepatozoon sciuri, sporogony of, in its squirrel flea vectors in England, 419

Hyalophora cecropia (Lepidoptera), hatching and size variation of spores of a species of *Nosema* (Microsporidia) in, 113

Ixodes, new species of, from Eastern Africa, 38
Ixodes, diomedeae n.sp., description of the female of, from *Diomedea chlororhynchus* and differentiation from *Ixodes rothschildi*, 124
oldi, Nuttall, 1913, description of the male and nymph of, 38
Ixodes, rothschildi, description of, from the burrows of *Fratercula arctica grabae*, 124

Leidynema appendiculata (Leidy, 1850), digestion in, 437

Manx shearwater, host of *Ixodes rothschildi*, 124
Mattesia orchopiae n.sp., a schizogregarine parasitic in a flea of squirrels in England, 375
Melophagus ovinus L., dissemination potential of, 353
acquirement of, by the lamb from its mother, 364
metacercariae, brachylaemid, effects of, on *Milax sowerbii* Férussac and *Agriolimax reticulatus* Müller, 261
Metachloridine, development of resistance in clones of *Plasmodium gallinaceum* to, 210
Meteterakis Karve, 1930, revision of the genus, 9
Milax sowerbii Férussac, effects of metacercariae of Brachylaemidae on, 261
infestation of, with metacercariae of Brachylaemidae, 303

Nematode parasites, of cockroaches, 433, 437
of sheep on lowland farms,
effects of anthelmintics on, 235
postparturient rise of populations of, 243
relation of behaviour of sheep to infection with, 251
revision of the genus *Meteterakis* Karve, 1930, 9
Nosema (Microsporidia), structure, hatching and size variation of the spores of, in *Hyalophora cecropia* (Lepidoptera), 113

Orchopeas wickhami, a new schizogregarine, *Mattesia orchopiae*, n.sp. in, 375
as vector of *Hepatozoon sciuri*, 419
Oxygen uptake, of *Trypanosoma lewisi* and *Trypanosoma equiperdum*, effect of some metabolic inhibitions on, 165
of *Trypanosoma lewisi* and *Trypanosoma equiperdum*, with especial reference to oxygen consumption in the presence of amino-acids, 149

Paryphostomum mehrai (Faruqui), egg and miracidium of, 96
Plasmodium gallinaceum, development of resistance to metachloridine in clones of, 210
Pterygosoma (Acarina: Pterygosomidae), notes on three species of, from Gerrhosaurid lizards, 89

Respiration, of *Trypanosoma brucei*, effect of immune sera on, 463

Salmo, salar L., host of *Crepidostomum metoecus* (Braun) and *C. farionis* (Müller) in Britain, 336
trutta L., host of *Crepidostomum metoecus* (Braun) and *C. farionis* (Müller) in Britain, 336
Sea birds, review of some Ticks (Acarina) of, 124
Sheep,
nematode parasites of,
effect of anthelmintics on, 235
postparturient rise of populations of, 243
behaviour of, in relation to nematode infections, 251
Sheep, and goats in Egypt, infection of, with *Globidium gilruthi* (Chatton, 1910), 291
Sheep ked, dissemination potential of, 353
acquirement of, by the lamb from its mother, 364
Sphaerostoma bramae (Müller) Lühe, the digenetic trematode, gametogenesis of, 293

Squirrel, flea of (*Orchopeas wickhami*), in England, as host of the schizogregarine, *Mattesia orchopiae* n.sp., 375
as vector of *Hepatozoon sciuri*, 419
Syngamus, species of, found in the intestine of *Turdus pilaris*, 121

Ticks (Acarina), some species of, found on sea birds, 124
Tipula paludosa, early stages of infection of the fatbody of the larva of, with tipula iridescent virus, 459
Tissue cultures, infected with *Trypanosoma cruzi*, electron microscopic study of thin sections of, 1
Toxocara canis, development of, in the dog, 184
Trematodes, cytological and histochemical criteria for evaluating development of, *in vivo* and *in vitro*, 131
Trypanosoma, brucei, effect of immune sera on the respiration of, 463
Trypanosoma, cruzi, electron microscopic study of, in thin sections of infected tissue cultures and in blood-agar forms, 1
Trypanosoma, equiperdum, oxygen uptake of, with especial reference to oxygen consumption in the presence of amino-acids, 149
oxygen uptake of, effect of some metabolic inhibitions on, 165
Trypanosoma, lewisi, oxygen uptake of, with especial reference to oxygen consumption in the presence of amino-acids, 149
oxygen uptake of, effect of some metabolic inhibitions on, 165
Turdus pilaris, host of a species of *Syngamus*, 121
Turkey poult, life history and pathogenic effects of *Eimeria adenoeides* in, 70

Virus, tipula iridescent, early stages of infection of the larva of *Tipula paludosa* with, 459

Vol

10.5
P22
v. 48
no. 3/4

JUL

AM

Issued

Vol. 48, Nos. 3, 4 November 1958

al Library
10.5
P22
v. 48
no 3/4

PARASITOLOGY

(Founded by G. H. F. Nuttall)

EDITED BY

D. KEILIN and P. TATE

THE UNIVERSITY OF MICHIGAN
JUL 7 1964
MEDICAL LIBRARY

IN CONJUNCTION WITH

A. BISHOP
F. J. BROWN
E. HINDLE
C. A. HOARE
G. LAPAGE
K. M. SMITH

CAMBRIDGE UNIVERSITY PRESS
BENTLEY HOUSE, 200 EUSTON ROAD, LONDON, N.W. 1
AMERICAN BRANCH: 32 EAST 57TH STREET, NEW YORK 22, N.Y.

Fifty-five Shillings net
(U.S.A. $9.50)

Issued 14 *November* 1958

UNIVERSITY OF MICHIGAN
MEDICAL LIBRARY

A list of the

JOURNALS

Published by the Cambridge University Press, may be had from

THE MANAGER
CAMBRIDGE UNIVERSITY PRESS
BENTLEY HOUSE
200 EUSTON ROAD
LONDON, N.W. 1

MICHROME STAINS

and Reagents for

BIOLOGY & HISTOCHEMISTRY

Adenosine	Giemsa Stain	Pyronin
Adenylic acids	Janus Green, B	Ribonuclease
Carminic Acid	Leishman Stain	Sudan Black
Celloidin	Luxol Fast Blue	Toluidine Blue
Gallocyanin	Methyl Green	Urease

OPTOIL: synthetic, non-sticky immersion oil.

CRISTALITE & CLEARMOUNT: synthetic, colourless, neutral mountants.

Service, uniform high quality, reliability, and immediate delivery

NOW READY: new books by Edward Gurr:

"Methods of Analytical Histology & Histochemistry", about 350 pages, Royal 8vo, price about 70/-.

"Microscopic Staining Techniques", No. 4 (1st edit.), 66 pages, price 6/- ($1.00 in U.S.A.), No. 3 (2nd edit.), 66 pages, 6/- ($1.00 in U.S.A.).

"A Practical Manual of Medical & Biological Staining Techniques", 460 pages, 42/-, Postage 1/6.

EDWARD GURR, LTD.

42 Upper Richmond Road West
LONDON, S.W.14

Publications issued by

COMMONWEALTH INSTITUTE OF ENTOMOLOGY

56 Queen's Gate, London, S.W. 7

BULLETIN OF ENTOMOLOGICAL RESEARCH

Published quarterly and containing original articles on Economic Entomology. *Issued Post Free.*
Annual Subscription (payable in advance) 100s. 0*d.*
Prices of back parts and volumes on application.

REVIEW OF APPLIED ENTOMOLOGY

Consisting of abstracts or reviews of current literature on Economic Entomology throughout the world. Published monthly in two series:

Series "A" dealing with insect and other Arthropod pests of cultivated plants, forest trees and stored products of animal and vegetable origin.

Series "B" dealing with insects, ticks, etc. conveying disease or otherwise injurious to man and animals. *Issued Post Free.*

	Series "A"	*Series "B"*
Annual Subscription (payable in advance)	60s. 0*d.*	30s. 0*d.*

Prices of back parts and volumes on application.

ZOOLOGICAL RECORD, part INSECTA

Published annually about October each year and containing as complete a record as possible of the literature of the previous year, chiefly from the systematic standpoint.
Annual Subscription (including postage) 51s. 0*d.*
Prices of back volumes on application.

Parasit

The Journal of General Microbiology

Editors: B. C. J. G. KNIGHT and A. F. B. STANDFAST

Contents Volume 19, No. 3, December 1958

UDEN, N. VAN, CARMO SOUSA, L. DO and FARINHA, M. On the intestinal yeast flora of horses, sheep, goats and swine.

BAIRD-PARKER, A. C. and DAVIS, G. H. G. The morphology of *Leptotrichia* species.

TAVERNE, J., MARSHALL, J. H. and FULTON, F. The purification and concentration of viruses and virus soluble antigens on calcium phosphate.

HOBSON, P. N., MANN, S. O. and OXFORD, A. E. Some studies on the occurrence and properties of a large Gram-negative coccus from the rumen.

MILLS, R. F. N. The effect of infecting the cells of the de-embryonated egg with influenza virus on their uptake of glucose and amino acids.

BERGERSEN, F. J. and BRIGGS, M. J. Studies on the bacterial component of soybean root nodules: cytology and organization in the host tissue.

SUNDMAN, V. and AF BJÖRKSTEN, K. The globular involution forms of the bifid bacteria.

KERRIDGE, D. The effect of actidione and other antifungal agents on nucleic acid and protein synthesis in *Saccharomyces carlsbergensis*.

HOPTON, J. W. The differential effect of temperature on gas production by a coliform organism.

MORGAN, W. J. B. The sensitivity of *Vibrio fetus* to Streptomycin and the emergence of resistant mutants.

CATLIN, B. W. and CUNNINGHAM, L. S. Studies of extracellular and intracellular bacterial deoxyribonucleic acids.

PERKINS, F. T. A ready reckoner for the calculation of geometric mean antibody titres.

SACKS, L. E. and PENCE, J. W. The influence of pH on the antibacterial action of subtilin A.

SNEATH, P. H. A. and COWAN, S. T. An electro-taxonomic survey of bacteria.

NORRIS, K. P. and GREENSTREET, J. E. S. On the infra-red absorption spectrum of *Bacillus megaterium*.

MENNA, M. E. DI. *Torulopsis ingeniosa* n.sp., from grass leaves.

WILLIAMSON, G. M. Dihydrostreptomycin and anaerobiosis—indirect evidence for two sites of action of dihydrostreptomycin.

SCHAECHTER, M., MAALØE, O. and KJELDGAARD, N. O. Dependency on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium*.

KJELDGAARD, N. O., MAALØE, O. and SCHAECHTER, M. The transition between different physiological states during balanced growth of *Salmonella typhimurium*.

OXFORD, A. E. The nutritional requirements of rumen strains of *Streptococcus bovis* considered in relation to dextran synthesis from sucrose.

SCOTT, W. J. The effect of residual water on the survival of dried bacteria during storage.

GROMAN, N. B. and MEMMER, R. Lysogeny and conversion in mitis and mitis-like *Corynebacterium diphtheriae*.

The subscription rate is 90s. net per volume of 3 parts. Single parts 35s. each, plus postage. Two volumes will be issued each year. Orders should be sent to

CAMBRIDGE UNIVERSITY PRESS
BENTLEY HOUSE, 200 EUSTON ROAD, LONDON, N.W.1

PARASITOLOGY is published in double issues twice a year. The numbers afterwards are issued in volumes each containing four numbers.

Papers for publication should be sent to Professor D. KEILIN, Molteno Institute, Downing Street, Cambridge. Other communications should be addressed to the University Press, Cambridge.

Papers forwarded to the Editors for publication are understood to be offered to *PARASITOLOGY* alone, unless the contrary is stated.

Contributors receive fifty copies of their papers free. Additional copies, not exceeding one hundred (except in special cases), may be had at cost price: these should be ordered when the final proof is returned.

The subscription price, payable in advance, is £5 net per volume, post-free (U.S.A. $17.00), double numbers 55*s*. net, postage extra (U.S.A. $9.50). Subscriptions may be sent to any Bookseller, or to The Cambridge University Press, Bentley House, 200 Euston Road, N.W. 1.

Enquiries from the U.S.A. should be addressed to the American Branch of the Press at 32 East 57th Street, New York, 22.

CONTENTS

(*All rights reserved*)

PAGE

CROFTON, H. D. Nematode parasite populations in sheep on lowland farms. IV. The effects of anthelmintic treatment. (With 7 Figures in the Text) . . . 235

CROFTON, H. D. Nematode parasite populations in sheep on lowland farms. V. Further observations on the post-parturient rise and a discussion of its significance. (With 6 Figures in the Text) 243

CROFTON, H. D. Nematode parasite populations in sheep on lowland farms. VI. Sheep behaviour and nematode infections. (With 2 Figures in the Text) . . . 251

FOSTER, R. The effects of trematode metacercariae (Brachylaemidae) on the slugs *Milax sowerbii* Férussac and *Agriolimax reticulatus* Müller. (With Plate X) . 261

PATERSON, NELLIE F. External features and life cycle of *Cucumaricola notabilis* nov. gen. et sp., a copepod parasite of the holothurian, *Cucumaria*. (With 11 Figures in the Text) 269

SOLIMAN, K. N. *Globidium gilruthi* (Chatton, 1910) infection in the digestive tract of sheep and goats in Egypt 291

GRESSON, R. A. R. The gametogenesis of the digenetic trematode *Sphaerostoma bramae* (Müller) Lühe. (With Plates XI and XII) 293

FOSTER, R. Infestation of the slugs *Milax sowerbii* Férussac and *Agriolimax reticulatus* Müller by trematode metacercariae (Brachylaemidae). (With 2 Figures in the Text) 303

ERASMUS, DAVID A. Studies on the morphology, biology and development of a strigeid cercaria (*Cercaria X* Baylis 1930). (With Plate XIII and 6 Figures in the Text) 312

THOMAS, J. D. Studies on *Crepidostomum metoecus* (Braun) and *C. farionis* (Müller), parasitic in *Salmo trutta* L. and *S. salar* L. in Britain. (With 10 Figures in the Text) 336

TETLEY, J. H. The sheep ked, *Melophagus ovinus* L. I. Dissemination potential . 353

TETLEY, J. H. The sheep ked, *Melophagus ovinus* L. II. Keds acquired by a lamb from its mother. (With 5 Figures in the Text) 364

DASGUPTA, BISWAPATI. A new schizogregarine, *Mattesia orchopiae* n.sp., in a flea of squirrels in England. (With 14 Figures in the Text) 375

ROTHSCHILD, MIRIAM. The bird fleas of Fair Isle. (With 10 Figures in the Text) . 382

JAIN, G. P. On *Cercaria mehrai* Faruqui, 1930 with notes on its life-history. (With 4 Figures in the Text) 413

DASGUPTA, B. and MEEDENIYA, KAMANI. The vector of *Hepatozoon sciuri* . (With 7 Figures in the Text) 419

MCCONNACHIE, ELSPETH W. Factors affecting the growth and behaviour of axenic strains of *Entamoeba invadens* Rodhain, 1934 423

LEE, D. L. On the morphology of the male, female and fourth-stage larva (female) of *Hammerschmidtiella diesingi* (Hammerschmidt), a nematode parasitic in cockroaches. (With 8 Figures in the Text) 433

LEE, D. L. Digestion in *Leidynema appendiculata* (Leidy, 1850), a nematode parasitic in cockroaches. (With 4 Figures in the Text) 437

TATCHELL, R. J. The physiology of digestion in the larvae of the horse bot-fly, *Gasterophilus intestinalis* (de Geer). (With 8 Figures in the Text) . . . 448

SMITH, KENNETH M. A study of the early stages of infection with the tipula iridescent virus. (With Plates XIV–XVII) 459

THURSTON, JUNE P. The effect of immune sera on the respiration of *Trypanosoma brucei in vitro*. (With 2 Figures in the Text) 463

REES, GWENDOLEN. A comparison of the structure of the scolex of *Bothriocephalus scorpii* (Müller 1776) and *Clestobothrium crassiceps* (Rud. 1819) and the mode of attachment of the scolex to the intestines of the host. (With Plates XVIII and XIX and 31 Figures in the Text) 468

BAILEY, L. The epidemiology of the infestation of the honeybee, *Apis mellifera* L., by the mite *Acarapis woodi* Rennie and the mortality of infested bees. (With 5 Figures in the Text) 493

Index of Authors 507

Index of Subjects 509

Printed in Great Britain at the University Press, Cambridge
(*Brooke Crutchley, University Printer*)

4538